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FEEDING AND POPULATION STUDIES ON
SOME BLOOD-SUCKING INSECTS

by

© MONIB SAYED TAWFIK

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Feeding and population studies on some blood-sucking insects" submitted by Monib Sayed Tawfik in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

In the stages of Cimex lectularius L. and Pediculus humanus L., and in adult female Aedes aegypti (L.) the weight of the blood meal was greater than the body weight. In C. lectularius L. and P. humanus L. the negative pressure required to draw the blood to the cibarial pump decreased from the first to the second instar and then increased to a maximum in the adult. This pressure may be as high as 5.6×10^5 dyne/cm^2. In A. aegypti female it is smaller than in any instar of C. lectularius or P. humanus. The muscular tension of the cibarial pump dialators in the species studied ranged from 18.4 to 5.7×10^3 g/cm^2 and the power output ranged from 13.3 to 2.75×10^3 g cm/sec/g of muscle. Sensilla were found in the cibarium of C. lectularius but not of P. humanus.

Successive small blood meals can induce moulting in C. lectularius but the interval between meals is very important in this effect. The food conversion efficiency in the different instars varies between 25.6 and 37%. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%. Unfed females do not lay eggs and the number of eggs laid per female as well as longevity showed good correlation with the amount of blood ingested. Egg viability is affected by the quantity of blood taken by males.

In C. lectularius, population density affects fecundity and the duration of the nymphal stadia, the preoviposition period, and of life, apparently through a contact stimulus. The effects reach optima at a population density of 4 to 8 insects/cm^2.

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PART I. STUDIES ON THE FEEDING OF SOME BLOOD-SUCKING INSECTS

1.0. Introduction

Blood-sucking insects can ingest enormous meals in a short time. In view of the narrowness of the feeding canal and relatively high viscosity of vertebrate blood, the rate of feeding, the negative pressure produced in the cibarial pump, and the power required from the cibarial pump dilators are of interest. All the nymphal instars and the adult males and females of *Cimex lectularius* L. and *Pediculus humanus* L., as well as adult female of *Aedes aegypti* (L.) were studied.

The structure and many details in the mechanism of the sucking apparatus in Hemiptera have been studied by Weber (1928, 1928a, 1929), Dickerson and Lavoipierre (1959), and Lavoipierre et al. (1959). Kemper (1932) and Snodgrass (1935, 1944) described in detail the feeding apparatus in the bedbug *C. lectularius*.

The anatomy of the mouth parts of some mosquitoes has been described in detail by Vogel (1921), Robinson (1939), Gordon and Lumsden (1939), Christophers (1960), Schiemenz (1957), and Clements (1963). The weight of the blood ingested by the females of different species of mosquitoes and the rates of ingestion have been reported in many publications. Fulleborn (1908) reported that the average amount of the blood taken by gorged female *A. aegypti* was 0.75 mg with a minimum and a maximum of 0.20 and 0.84 mg respectively. He also found that 53 out of 137 took a blood meal much greater than their own weight.

Jeffery (1956) recorded that Anopheles quadrimaculatus and Anopheles albimanus ingest about 3.46 and 2.58 mg of blood respectively. Christophers (1960) reported that the feeding canal in Aedes aegypti is about 2 mm long and has a diameter of 0.03 mm. He added that from 2 to 4 mm^3 of blood pass through this channel in 2 minutes and he claimed that the linear rate of flow is from 2 to 4 cm/sec.

The morphology of the piercing organs in Siphunculata has been dealt with by Cholodkowsky (1904), Enderlein (1905, 1905a), Pawlowsky (1906), Harrison (1914), Sikora (1916), Peacock (1918), Florence (1921), Vogel (1921), Fernando (1933), Snodgrass (1944) and Stojanovich (1945). Summarized accounts are also given by Patton and Evans (1929), Metcalf and Flint (1962), and Imms (1960). The relationships between food supply and the biology of Pediculus humanus have been described by Nuttall (1917b), Buxton (1947), Busvine (1948) and Gooding (1963).

Accounts of the biology of Cimex lectularius, reared under various conditions, have been published by Bacot (1914), Hase (1917, 1919, 1930), Cragg (1923), Jones (1930), Kemper (1930), Janisch (1933, 1935), Kassianoff (1937), Johnson (1939, 1940a, 1940b, 1942), de Meillon and Golberg (1946, 1947), and Bell and Schaefer (1966). More recently Usinger (1966) published a monograph of Cimicidae reviewing most of the work done on this group of insects. Similarly studies on fertilization and physiology of reproduction of the adults have been published by Cragg (1915, 1920), Mellanby (1935, 1939a)

and Davis (1964, 1965a, 1965b). Although a relationship between food supply and the growth of C. lectularius has been demonstrated (Titschack 1930 and de Meillon and Golberg 1946 and 1947), the effect of the size and frequency of blood meals has not been studied. This investigation was undertaken with a view to filling some gaps in the knowledge of bedbug biology.

1.1. Feeding apparatus and feeding mechanisms

Whole mounts of the head and mouth parts were prepared. The insects were soaked overnight in 5% potassium hydroxide solution, washed thoroughly in water, stained with acid fuchsin if necessary, dehydrated in ethanol, cleared in xylol and mounted in Canada balsam. Serial cross sections of the head were necessary and these were cut at 6 microns and stained with Mallory's triple stain. Measurements were made with an ocular micrometer.

The labium of the bed-bug C. lectularius (Figure 1) is considerably longer than the head and when not in use is turned backwards at its base with its distal part between the fore legs. It is four segmented with the first segment mostly concealed by the labrum and the maxillary lobes. The labium is grooved on the lower surface and the sides diverge near the tip of the last segment to leave a small aperture. The maxillae and the mandibles are stylet like and are held together within the groove of the labium. Between the maxillary stylets is a minute salivary duct and a relatively large food canal. The length of the food canal and its radius vary from one instar to the next (Table 2, page 23.). At the base of the labium the maxillae turn backwards into the head pouches and diverge against the base of the hypopharynx where the food canal opens into the anterior surface of the hypopharyngeal lobe that leads back into the cibarial pump. The hypopharynx is a small lobe and

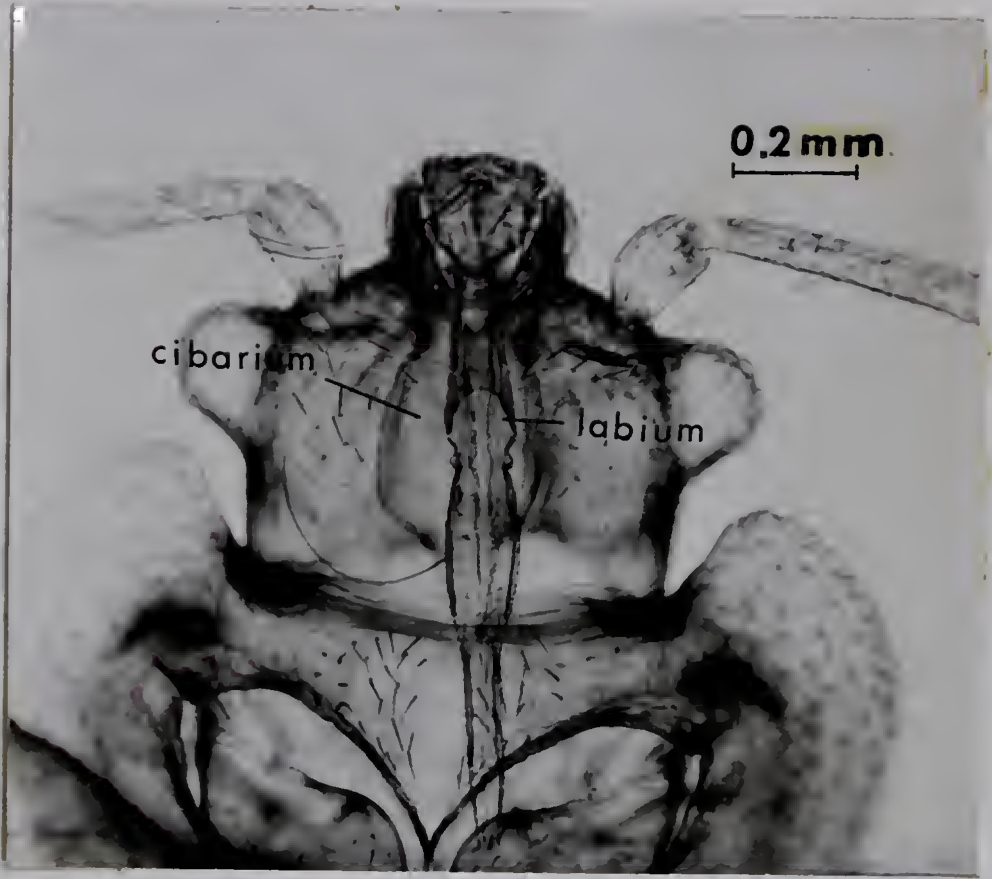


Fig. 1. Head and mouth parts of female C. lectularius (ventral view).

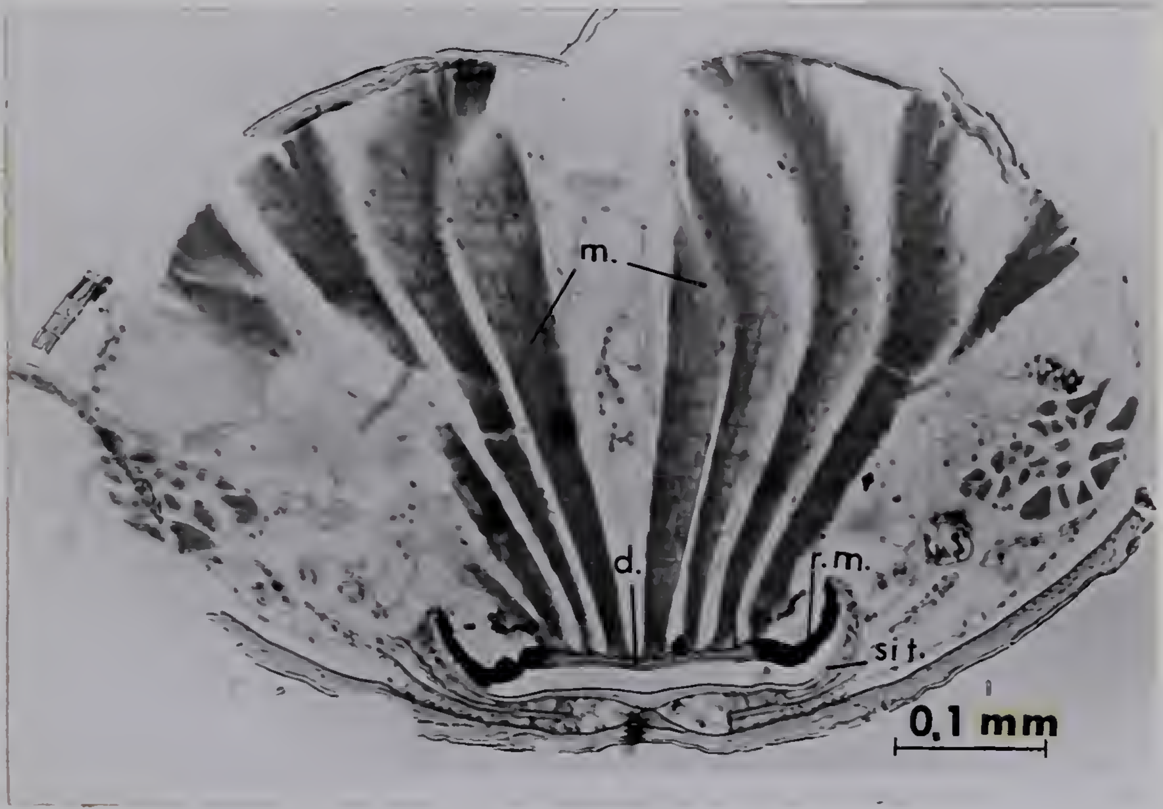


Fig. 2. Cross section through the head of female C. lectularius. (d) diaphragm; (rm) rubbery margin; (m) cibarial pump dilators.

its dorsal surface is continued back into a large and deeply concave sitophore with strong lateral margins, which is the floor of the cibarial pump (Figure 2). The dorsal wall of the cibarial pump is in the form of a diaphragm (d.) which is attached by a rubbery margin (r.m.) along either edge of the sitophore (sit.). The diaphragm when not in action is completely collapsed into the cavity of the sitophore. The measurements of the various parts of the cibarial pump in the different instars are shown in Tables 3 and 4 (Pages 26 and 29). The dilator muscles (m.) consist of two lateral bundles of fibers, arising on nearly the whole clypeus. Measurements of these muscles are shown in Tables 3 and 4 (Pages 26 and 29). The cibarial pump discharges directly into the tubular oesophagus as there is no differentiated pharynx.

When the bed-bug feeds the stylets are forced into the skin of the host. The labium helps in lowering the head of the insect near the skin of the host by a backward bending of the third and fourth segments. The mandibular stylets are the effective piercing organs while the two maxillary stylets closely adhere to act as a sucking needle. Contraction of the cibarial pump dilators moves the diaphragm upwards during the filling stroke. Emptying is achieved by the return of the diaphragm under the elastic force of the rubbery margins.

The mouth parts of female _A_. _aegypti_, like those of _C_. _lectularius_, are modified for piercing and most of the mouth parts are extended into long and slender stylets. The labium

is relatively stout and contains a dorsal groove in which the stylets lie closely beside each other in a fascicle. The labium ends with a pair of labella. The labrum is a broad pointed stylet in the labial groove. It is curved so that its edges meet and thus forms the food canal. Towards the base of the labrum the edges separate and the hypopharynx forms the floor of the food canal. The average length and the radius of the food canal are shown in Table 2 (Page 23). The hypopharynx is a very slender stylet with a median rib containing the salivary canal. The mandibles are also stylets which come to a simple point without teeth at the tip. The maxillae, which are the principal piercing organs, each consist of a long flattened stylet with a curved end. The cibarial pump (cb.), Figure 3, is located under the clypeus (cl.) and is trough-shaped. The lower wall of the trough is well chitinized and is continuous with the upper surface of the hypopharynx. The upper wall is thinner and is continuous with the inner surface of the labrum. The upper wall is elastic and when the cibarial pump is not in action it lies close to the lower wall. Attached to the upper wall of the cibarial pump are two bundles of muscles, the dilators of the cibarial pump (m.), which have their origin from the clypeus. Measurements of the cibarial pump and its dilators are shown in Tables 3 and 4 (Pages 26 and 29). For a short distance behind the cibarial pump the cuticle is thin and forms a valve. The cibarial pump is connected to the pharyngeal pump by a narrow canal.

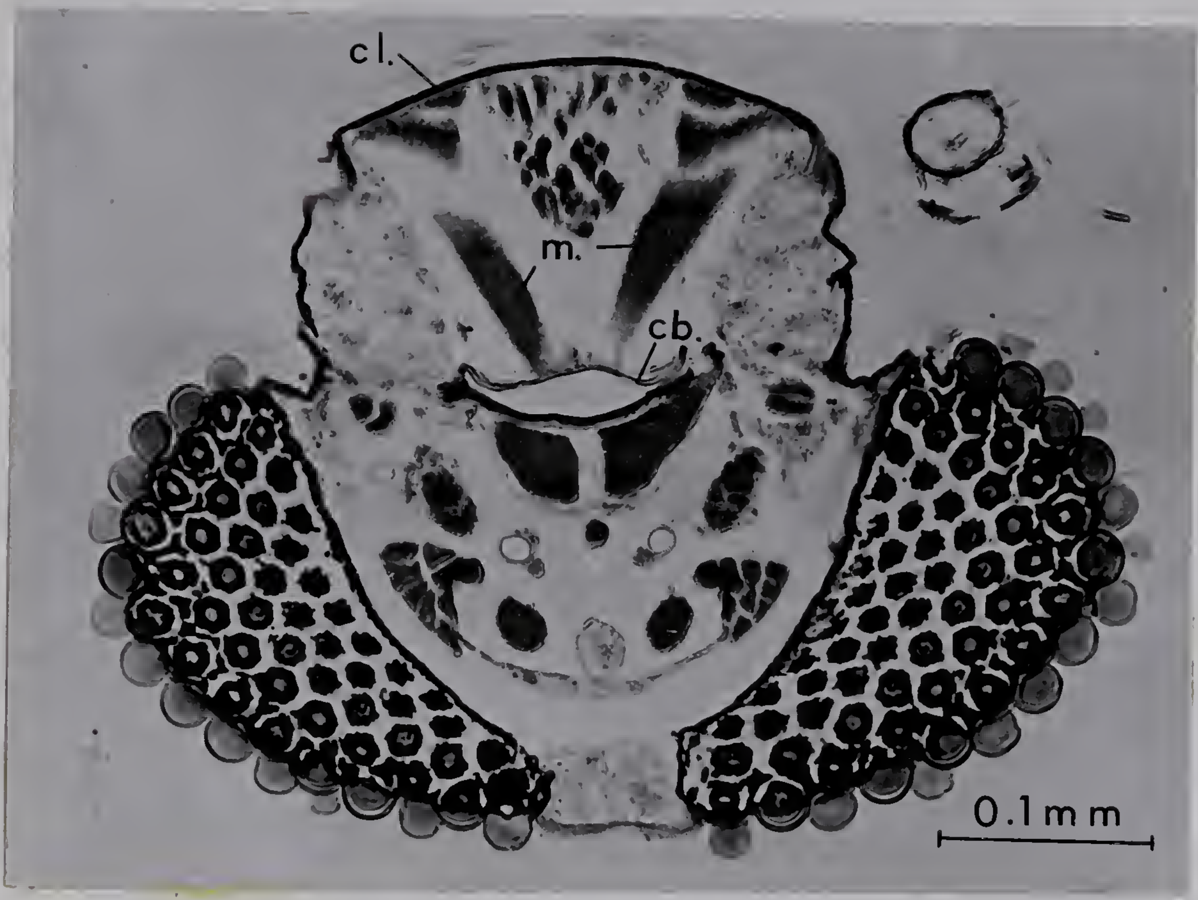


Fig. 3. Cross section through the head of female _A. aegypti_. (cb) cibarial pump; (cl) clypeus; (m) cibarial pump dilators.

The head of P. humanus is produced anteriorly into a very small snout-like proboscis probably formed from the labrum and in which is a terminal opening or prestomum. This snout-like proboscis is armed internally with small teeth used to grip the host during feeding. The prestomum opens to a preoral cavity within the head, the upper part of it is continuous with the cibarial pump; the lower part extends to a long sac which contains the stylets and nearly reaches the posterior end of the head. The three stylets lie one above the other, the dorsal and ventral ones forked at their bases. The dorsal stylet is made of two united halves with the edges curved upwards and rolled over each other to form the food canal. The ventral stylet is the effective piercing organ and its apex is armed with teeth. The median stylet is pierced throughout its length by the salivary duct. The homology of these stylets with the mouth parts of ~~biting~~ chewing insects has been a subject of dispute for many years. Schölzel (1937) reinvestigated the development of these mouth parts. He claimed that the ventral stylet is the labium, and he said that the maxillae and mandibles are reduced and merged into the lateral walls of the preoral cavity and that the dorsal and median stylets are both derived from the embryonic hypopharynx. This interpretation was accepted by Snodgrass(1944). During feeding the blood passes through the food canal of the dorsal stylet into a trough that fits into the proximal end of the food canal. This trough, which is closed dorsally by the inner clypeal wall, is connected with the cibarial pump. Figure 4 shows the action of

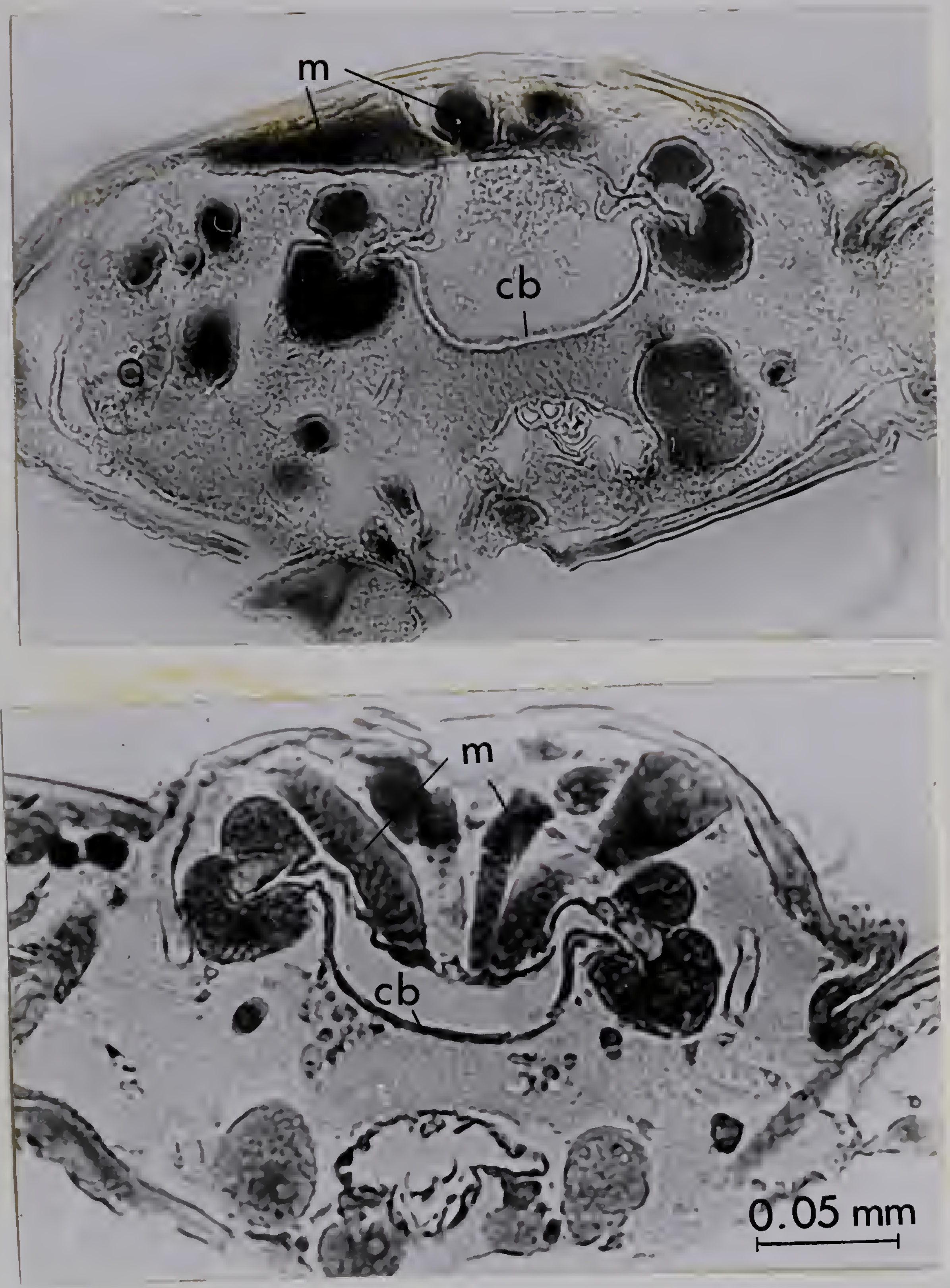


Fig. 4. Cross section through the head of the female P. humanus. A. During filling stroke. B. During emptying stroke. (cb) cibarial pump; (m) cibarial pump dilators.

the cibarial pump during the filling and emptying strokes. The measurements of the feeding canal and the various parts of the cibarial pump are shown in Tables 3 and 4 (Pages 26 and 29).

1.2. Sense organs in the food canal

von Gernet and Buerger (1966) studied the labral and cibarial sense organs in some mosquitoes. They found two types of sense organs on the labra of female mosquitoes, four hair sensilla at the tip and two subapical sensilla. These labral sense organs were found to be innervated by a branch of the labral nerve. Two groups of sense organs were also found in the cibarium. A dorsal group of four types of sensilla innervated by a second branch of the labral nerve and a ventral group at the posterior end of the cibarium innervated by a small branch of the fronto-labral nerve. They suggested that food is detected by the apical labral sense organs and the pumping action is initiated by impulses received by the cibarial muscles from the labral sense organs via the frontal ganglion. They also suggested that the cibarial sense organs control the openings of the stomach and diverticula, thus setting the food-directing mechanism in action.

Similarly, I studied _C. lectularius_ and _P. humanus_. No sense organs could be located on the maxillae of _C. lectularius_. On the other hand, two types of sensilla were found in the cibarium. A dorsal group consists of six sensilla at the anterior end, three on each side of the cibarium (Figure 5A). These are formed from minute hollow spines and innervated by fine dendrites originating from the adjoining sensory cells. These sensory cells seem to be innervated by a branch from the frontal nerve. The second group consists of two campaniform

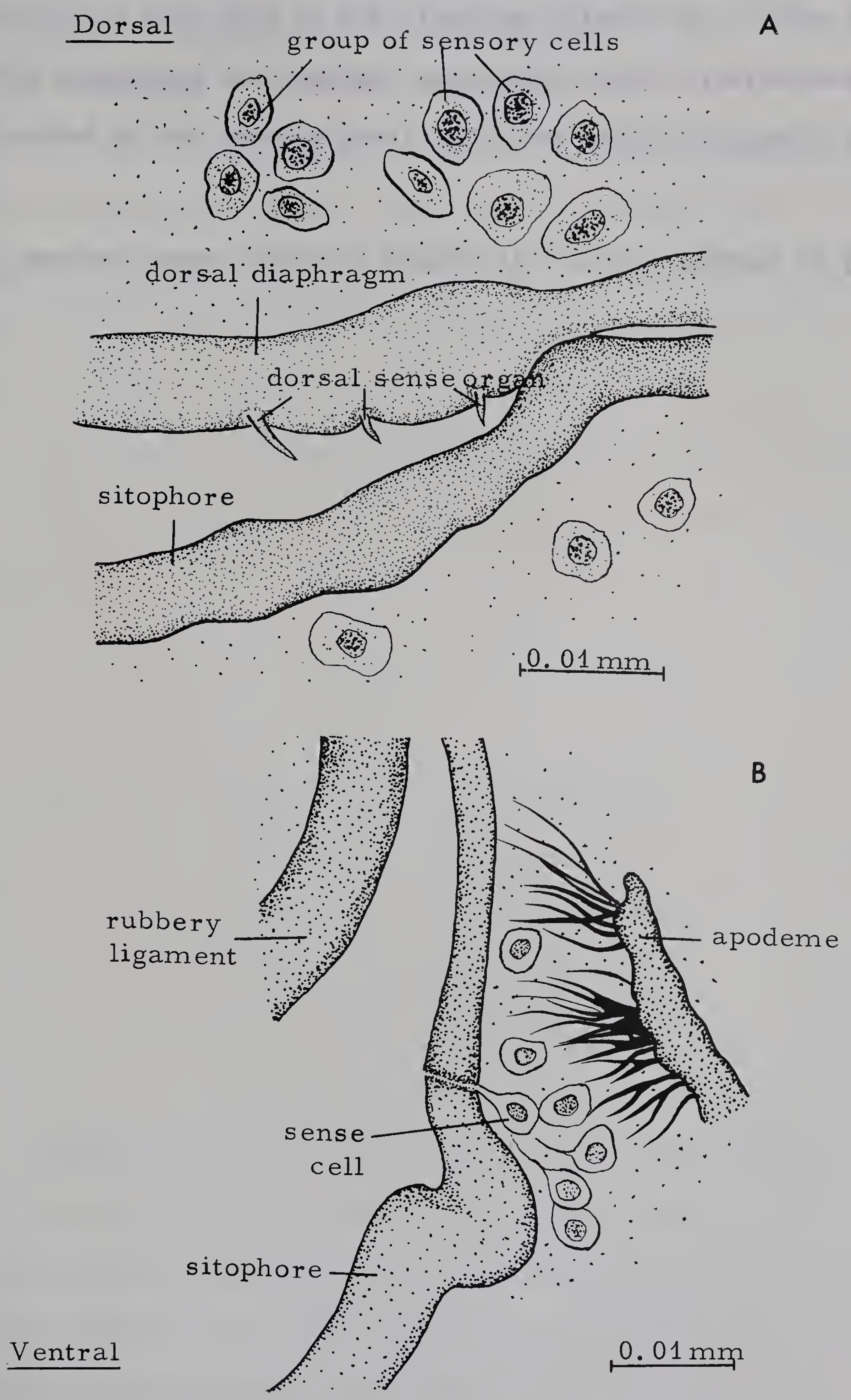


Fig. 5. Sense organs in the cibarium of female
C. lectularius. A. dorsal sense organs
B. sitophore sense organs

[illegible]

sensilla, one on each side of the sitophore (Figure 5B). Miles (1958) found that Oncopeltus and Dindymus versicolor, could discriminate between liquids sucked up the feeding canal while any contact receptors were masked.

No sensory organs could be located in the food pathway of P. humanus.

1.3. Rate of feeding and the forces involved

1.3.0. Methods

Feeding was observed under a binocular microscope and the feeding period was timed with a stop watch. The weight of the blood meal was determined by weighing the insects on a torsion balance before and after feeding. In all the experiments the insects were fed naturally on human blood.

Calculation of the pressure difference required to force a liquid along a tube at known rate can be made from Poiseuille's formula:

$$\Delta P = \frac{8\,Q\,l\,\eta}{\pi\,r^4\,t}$$

where ΔP = pressure difference in dynes/cm^2

Q = volume moved in cm^3

η = viscosity of the liquid in poises

l = length of the tube in cm

r = radius of the tube in cm

t = time in seconds.

Poiseuille's formula was applied, for the determination of the negative pressure required to draw the blood to the cibarial pump in these insects, on the assumption that the blood behaves like a simple fluid and the flow is laminar, because no more precise procedure has been developed. These assumptions are questionable.

The volume of the blood (Q cm^3) ingested during feeding for t seconds was determined from the weight, taking the density of

the human blood as 1.056 g/cm^3 (Spector 1956, P. 51). The viscosity of human blood is approximately 0.025 poises at 38 C (Mitchell 1948, P. 407). The pressure difference required for the observed flow rate was obtained by applying Poiseuille's formula. The negative pressure in the cibarium was obtained by subtracting from the pressure difference the capillary blood pressure which is 12 mm Hg or 1.6×10^4 $dynes/cm^2$ (Wright 1952). The muscular force required to provide this negative pressure was calculated by multiplying the corrected pressure difference by the area of the diaphragm.

The force obtained from these calculations was doubled for the estimation of the tension of the cibarial pump dilators on the assumption that half of the time is spent in the filling stroke. The muscular tension per unit cross sectional area of muscles was obtained by dividing the corrected force by the mean cross sectional area of the cibarial pump dilators. The mean cross sectional area was determined from cross sections of the cibarial pump dilators cut at 6 microns.

The power required of the cibarial pump dilators expressed as ergs/sec/g of muscle and g cm/sec/g of muscle was found by multiplying the force by the total working travel of the diaphragm in a second and dividing by the weight of the cibarial pump dilators. The total working travel was determined by multiplying the length of the rubbery margin by the number of pump cycles per second. The geometry of the cibarium, flat and expanded suggests that the diaphragm moves approximately the length of the rubbery margin in each stroke.

For determining the weight of these muscles the volume

was obtained by multiplying the mean cross sectional area by the length of muscles and assuming the specific gravity of insect muscle to be one.

1.3.1. Rate of feeding.

The rates of feeding observed are shown in Table 1.

The feeding period of *C. lectularius* and *A. aegypti* females was taken as the time required for the insect to feed till engorged and in *P. humanus* as the time required for the insect to feed till the blood started to exude from the anus.

The feeding period of *C. lectularius* is a minimum in the first nymphal instar, increases to a maximum in the fifth instar and then decreases in tha adult. The feeding period of the adult female is shorter than that of the male.

In *P. humanus* the feeding period of the first instar is slightly longer than that of the second instar and shorter than that of the adult. The feeding period of the female is shorter than that of the male. The feeding period in *P. humanus* is much longer than in *C. lectularius* and in *A. aegypti* female. The difference between the feeding period of *P. humanus* and both *C. lectularius* and female *A. aegypti* should be much greater than the estimated results in Table 1 because feeding in lice takes place for some time after the blood starts to exude from the anus. The duration of the feeding period can be correlated to the behaviour of these insects and their access to their hosts and a longer feeding period in *P. humanus* may be due to their living on the host while both *C. lectularius* and *A. aegypti* leave their host after feeding.

The feeding period of the female *A. aegypti* is longer than that of each one of the first four instars and shorter than that of the fifth instar and the adult of *C. lectularius*.

TABLE I - Feeding period, weight of blood meal, and rate of feeding in

C. lectularius, P. humanus, and A. aegypti female.

Species	Feeding period to engorgement in seconds	Weight of blood meal in milligrams	Ratio of weight of blood meal to body weight	Weight of blood taken/second in micrograms	Number of pump cycles/second	Weight of blood taken/pump cycle in micrograms
C. lectularius						
First	146.5±17.4* (20) 105 - 187	0.260±0.06* (20) 0.13 - 0.44	3.7	1.77±0.045* (20) 1.23 - 2.35	4.0±0.05* (40) 2.9 - 4.6	0.44
Second	156.8±4.2 (20) 129 - 190	0.62±0.06 (20) 0.22 - 0.92	4.5	3.95±0.15 (20) 1.63 - 4.84	3.8±0.04 (36) 3.2 - 4.5	1.03
Third	175.3±4.3 (25) 135 - 282	1.09±0.09 (25) 0.46 - 1.82	3.2	6.20±0.13 (25) 3.41 - 6.62	3.3±0.04 (28) 3.0 - 3.8	1.89
Fourth	234.4±8.8 (20) 135 - 282	2.86±0.09 (25) 1.14 - 3.01	4.4	11.73±0.14 (25) 8.44 - 12.04	3.3±0.02 (40) 2.9 - 3.6	3.58
Fifth ♀	305.6±4.1 (15) 240 - 422	5.64±0.35 (20) 2.21 - 8.42	4.9	18.46±0.48 (25) 9.21 - 19.98	3.0±0.03 (20) 2.7 - 3.3	6.18
Fifth ♂	290.0±5.9 (15) 240 - 310	5.42±0.31 (15) 2.64 - 8.10	4.8	18.69±0.76 (15) 11.00 - 26.2	3.0±0.03 (20) 2.8 - 3.3	6.29
Female	268.9±10.3 (19) 213 - 350	6.48±0.41 (15) 5.20 - 11.20	2.8	24.10±0.72 (15) 19.24 - 32.0	2.4±0.02 (30) 2.3 - 2.5	9.88

Cont'd.

TABLE I - (Cont'd.)

Species	Feeding period to engorgement in seconds	Weight of blood meal in milligrams	Ratio of weight of blood meal to body weight	Weight of blood taken/second in micrograms	Number of pump cycles/second	Weight of blood taken/pump cycle in micrograms
Male	286.9±14.6 (20) 195 - 410	6.23±0.41 (15) 3.90 - 11.4	2.8	21.70±0.46 (15) 20.86 - 27.91	2.4±0.02 (30) 2.3 - 2.5	8.89
P. humanus**						
Instar I	504±7.7 (15) 445 - 565	0.11±0.005 (15) 0.08 - 0.13	1.8	0.22±0.006 (15) 0.18 - 0.25	2.8±0.23 (15) 2.1 - 3.3	0.08
Instar II	443.1±9.2 (15) 275 - 720	0.21±0.007 (15) 0.15 - 0.30	1.4	0.48±0.008 (15) 0.40 - 0.53	5.3±0.03 (21) 5.0 - 5.6	0.09
Adult ♀	764.4±45.6 (20) 459 - 1372	1.13±0.078 (18) 0.82 - 1.80	1.2	1.48±0.027 (15) 1.34 - 1.80	4.6±0.001 (38) 3.9 - 5.0	0.32
Adult ♂	782.9±48.4 (15) 430 - 1210	0.95±0.07 (15) 0.50 - 1.40	1.1	1.21±0.016 (15) 1.14 - 1.31	4.2±0.06 (15) 3.7 - 4.5	0.29
A. aegypti						
Adult ♀	255.1±8.8 (15) 210 - 295	1.62±0.18 (15) 0.84 - 2.90	1.2	6.35±0.34 (15) 4.00 - 9.83	4.9±0.20 (15) 4 - 6	1.29

* Mean ± S.E.
(number of insects used)
Range

** Third nymphal instar in P. humanus was not studied because of some difficulties in maintaining the culture.

When the insects feed till engorgement the weight of the blood meal varies from one instar to the next. In *C. lectularius* and *P. humanus* the minimum value is found in the first nymphal instar and the maximum in the adult stage. The weight of the female's blood meal was greater than the male's in both *C. lectularius* and *P. humanus*. The weight of the blood meal in *C. lectularius* is greater than that in *P. humanus*. The weight of the blood meal in *A. aegypti* is greater than that of any instar in *P. humanus* and than that of the first three instars of *C. lectularius*, but smaller than that of the other instars in the latter species.

In all three species the weight of the blood meal is greater than the body weight. The ratio of weight of the blood meal to body weight is greater in *C. lectularius* than in the other two species. In *C. lectularius* and *P. humanus* the ratio is higher in the nymphal instars than in either the female or the male because of the flexibility of the integument which becomes more chitinized in the adult stage.

In *C. lectularius*, as shown in Table 1, the number of pump cycles per second decreases gradually from the first instar to the adult stage. In *P. humanus* the number of pump cycles per second increases from the first to the second instar and then decreases slightly in the adult stage.

The rate of feeding in *C. lectularius* and *P. humanus*, expressed as micrograms of blood taken per second or as micrograms of blood taken per pump cycle, increase gradually from the first

instar to the adult. The rate of feeding is greater in the adult female than in the male in both species. In the first nymphal instar of C. lectularius the rate of feeding is much greater than in any instar of P. humanus. The weight of blood taken per second by A. aegypti females is greater than that by the first three nymphal instars of C. lectularius and smaller than that taken by each of the other instars. On the other hand, the weight of blood taken per pump cycle in A. aegypti is greater than the first and second instars of C. lectularius and smaller than the other instars.

1.3.2. Negative pressure in the cibarial pump

Table 2 shows the negative pressure required to draw the blood to the cibarial pump at the observed rates in these insects. In *C. lectularius* the pressure difference decreases from the first to the second nymphal instar and increases from the second to the adult stage. The pressure difference in female *A. aegypti* is smaller than any instar of *C. lectularius* and than the first instar and the adult stage of *P. humanus*. In the last species it decreases from the first to the second nymphal instar with a maximum value in the adult. Table 2 also shows the results obtained by Bennet-Clark (1963) in the fifth nymphal instar of *Rhodnius prolixus* (Ståhl). He claimed that $1.96x10^{6}$ dynes/cm^{2} or about 2 atmospheres, can be taken as the likely minimum, but he ignored the proximal 5 mm of the feeding canal and assumed that the viscosity of the blood is equal to that of water. He added that if he took these into consideration the pressure required for *Rhodnius* to feed could be as high as 9 atmospheres i.e. $9.12x10^{6}$ dynes/cm^{2}.

TABLE 2 - Negative pressure (relative to atmospheric) in the cibarial pump in _C_. _lectularius_, female _A_. _aegypti_, _P_. _humanus_ and _R_. _prolixus_.

Species	Q/t cm^3/sec	$\overset{*}{l}$= length cm	$\overset{*}{r}$= mean radius cm	ΔP dyne /cm^2	ΔP ** corrected dyne/cm^2
_C_. _lectularius_					
First	$1.7x10^{-6}$	0.055 (20) 0.050-0.058	$4.4x10^{-4}$ (5) $4.1x10^{-4}$-$4.9x10^{-4}$	$1.6x10^5$	$1.4x10^5$
Second	$3.7x10^{-6}$	0.064 (15) 0.060-0.069	$6.0x10^{-4}$ (7) $5.8x10^{-4}$-$6.4x10^{-4}$	$1.1x10^5$	$9.4x10^4$
Third	$5.9x10^{-6}$	0.079 (15) 0.073-0.082	$6.6x10^{-4}$ (5) $6.5x10^{-4}$-$6.8x10^{-4}$	$1.6x10^5$	$1.4x10^5$
Fourth	$1.1x10^{-5}$	0.097 (15) 0.090-0.102	$6.8x10^{-4}$ (5) $6.7x10^{-4}$-$6.9x10^{-4}$	$3.3x10^5$	$3.1x10^5$
Fifth	$1.8x10^{-5}$	0.097 (15) 0.094-0.109	$7.0x10^{-4}$ (5) $6.8x10^{-4}$-$7.2x10^{-4}$	$4.6x10^5$	$4.4x10^5$
Female	$2.3x10^{-5}$	0.123 (12) 0.119-0.127	$8.0x10^{-4}$ (8) $7.7x10^{-4}$-$8.3x10^{-4}$	$4.4x10^5$	$4.2x10^5$
Male	$2.1x10^{-5}$	0.100 (10) 0.098-0.108	$7.0x10^{-4}$ (5) $6.8x10^{-4}$-$7.3x10^{-4}$	$5.6x10^5$	$5.4x10^5$
_A_. _aegypti_					
Female	$6.0x10^{-6}$	0.182 (20) 0.200-0.173	$1.1x10^{-3}$ (10) $9.9x10^{-4}$-$1.4x10^{-3}$	$4.6x10^4$	$3.0x10^4$
Cont'd.					

TABLE 2 - (Cont'd.)

Species	Q/t cm^3/sec	l^*= length cm	r^*= mean radius cm	Δ P dyne /cm^2	Δ P** corrected dyne/cm^2
P. humanus					
First	$2.1x10^{-7}$	0.027 (10) 0.024-0.031	$2.8x10^{-4}$ (5) $2.5x10^{-4}$-$3.2x10^{-4}$	$6.1x10^{4}$	$4.5x10^{4}$
Second	$1.7x10^{-7}$	0.033 (12) 0.032-0.034	$3.1x10^{-4}$ (6) $3.0x10^{-4}$-$3.4x10^{-4}$	$3.9x10^{4}$	$2.3x10^{4}$
Female	$1.4x10^{-6}$	0.040 (10) 0.038-0.043	$3.6x10^{-4}$ (5) $3.4x10^{-4}$-$3.9x10^{-4}$	$2.1x10^{5}$	$1.9x10^{5}$
Male	$1.1x10^{-6}$	0.040 (12) 0.037-0.042	$3.7x10^{-4}$ (5) $3.3x10^{-4}$-$3.9x10^{-4}$	$1.5x10^{5}$	$1.3x10^{5}$
R. prolixus					
Fifth***	$3.3x10^{-4}$	0.020	$5.0x10^{-4}$	$1.96x10^{6}$ $9.12x10^{6}$	

* Mean
(number of insects used)
Range

** Δ P "corrected" =ΔP - capillary blood pressure.

*** Data from Bennet-Clark (1963,Page 223).

1.3.3. The muscular tension and the power output of the cibarial pump dilators.

The results are shown in Tables 3 and 4. In *C. lectularius* the mean tension required of the cibarial pump dilators decreases from the first to the second nymphal instar and then increases to a maximum in the adult. The value for the cibarial pump dilators of the male was larger than that of the female. In the female *A. aegypti* the muscular tension per unit sectional area of the cibarial pump dilators is larger than that of the first three nymphal instars and smaller than that of the other instars of *C. lectularius*. In *P. humanus* the mean muscular tension of the cibarial pump dilators decreases from the first to the second nymphal instar and reaches a maximum in the adult with a higher value in the female than in the male.

From the data published by Bennet-Clark (1963) the mean muscular tension per cm^2 sectional area of the cibarial pump dilators of the fifth nymphal instar of *R. prolixus* was calculated after knowing the area of the diaphragm (effective piston) and the cross sectional area of the muscles. According to his data the muscular tension per unit sectional area was found to be in the range between 1.2 to 5.7 kg/cm^2.

The muscular tension of the cibarial pump dilators most probably reaches a peak value of two times the estimated mean in Table 3. Wigglesworth (1965) reported the maximum load a muscle can raise per square centimeter of cross section in some species and he found that there is no great difference between insects

TABLE 3 - The mean force and muscular tension of the cibarial pump dilators in C. lectularius, female A. aegypti, P. humanus, and R. prolixus.

Species	Area of the diaphragm* cm^2	Mean force** dynes	Mean muscular tension*** dynes	Cross sectional* area of muscles cm^2	Muscular tension/cm^2	
					dyne/cm^2	g/cm^2
C. lectularius						
First	$8.6x10^{-5}$ (5) $7.6x10^{-5}$-$9.4x10^{-5}$	12.0	24.0	$4.2x10^{-4}$ (5) $3.7x10^{-4}$-$4.8x10^{-4}$	$5.7x10^{4}$	58.1
Second	$1.0x10^{-4}$ (7) $9.6x10^{-5}$-$1.4x10^{-4}$	11.0	22.0	$5.1x10^{-4}$ (5) $4.7x10^{-4}$-$5.6x10^{-4}$	$4.3x10^{4}$	43.8
Third	$1.6x10^{-4}$ (5) $1.0x10^{-4}$-$2.1x10^{-4}$	22.4	44.8	$8.2x10^{-4}$ (6) $7.9x10^{-4}$-$8.4x10^{-4}$	$5.5x10^{4}$	56.1
Fourth	$2.5x10^{-4}$ (5) $2.2x10^{-4}$-$2.9x10^{-4}$	77.5	155.0	$1.2x10^{-3}$ (6) $1.0x10^{-3}$-$1.5x10^{-3}$	$1.3x10^{5}$	132.6
Fifth	$3.2x10^{-4}$ (5) $2.9x10^{-4}$-$3.7x10^{-4}$	140.8	281.6	$1.3x10^{-3}$ (5) $1.0x10^{-3}$-$1.8x10^{-3}$	$2.2x10^{5}$	224.3
Female	$5.7x10^{-4}$ (8) $4.9x10^{-4}$-$5.9x10^{-4}$	239.4	478.8	$2.1x10^{-3}$ (7) $1.9x10^{-3}$-$2.5x10^{-3}$	$2.3x10^{5}$	234.5
Male	$4.1x10^{-4}$ (5) $3.8x10^{-4}$-$4.5x10^{-4}$	221.4	442.8	$1.5x10^{-3}$ (8) $1.2x10^{-3}$-$1.9x10^{-3}$	$2.9x10^{5}$	295.7

Cont'd.

TABLE 3 - (Cont'd.)

Species	Area of the diaphragm* cm^2	Mean force** dynes	Mean muscular tension*** dynes	Cross sectional area of muscles* cm^2	Muscular tension/cm^2	
					dyne/cm^2	g/cm^2
A. aegypti						
Female	$1.9x10^{-4}$ (10) $1.4x10^{-4}-2.3x10^{-4}$	5.7	11.4	$1.8x10^{-4}$ (10) $1.3x10^{-4}-2.3x10^{-4}$	$6.3x10^{4}$	64.2
P. humanus						
First	$2.4x10^{-5}$ (6) $1.9x10^{-5}-2.7x10^{-5}$	1.1	2.2	$6.8x10^{-5}$ (5) $6.1x10^{-5}-7.6x10^{-5}$	$3.2x10^{4}$	32.6
Second	$4.5x10^{-5}$ (5) $3.9x10^{-5}-5.2x10^{-5}$	1.0	2.0	$1.1x10^{-4}$ (5) $9.4x10^{-5}-1.7x10^{-4}$	$1.8x10^{4}$	18.4
Female	$8.1x10^{-5}$ (8) $7.3x10^{-5}-9.2x10^{-5}$	15.4	30.8	$1.9x10^{-4}$ (8) $1.4x10^{-4}-2.5x10^{-4}$	$1.6x10^{5}$	163.1
Male	$6.4x10^{-5}$ (8) $5.8x10^{-5}-7.4x10^{-5}$	8.3	16.6	$2.0x10^{-4}$ (8) $1.3x10^{-4}-2.2x10^{-4}$	$8.3x10^{4}$	84.6
R. prolixus						
Fifth	$9.8x10^{-3}$ (2) $9.6x10^{-3}-1.0x10^{-2}$	$1.9x10^{4}$ # $8.9x10^{4}$	$3.8x10^{4}$ # $17.8x10^{4}$	$3.2x10^{-2}$ (2) $2.8x10^{-2}-3.5x10^{-2}$	$1.2x10^{6}$ # $5.6x10^{6}$	$1.2x10^{3}$ + $5.7x10^{3}$

* Average
(number of measurements)
Range

** Mean force = ΔPx area of the diaphragm

*** Mean muscular tension during working stroke = mean force x 2

# The two values correspond to the two different values of Δ P of Bennet-Clark (See Table 2).

and vertebrates as the value for man is 6 - 10 kg, for the frog 3 kg, for the mandibular muscles of insects 3.6 - 6.9 kg, for the hind leg of Tettigonia 4.7 kg and for the flexor tibia of Decticus 5.9 kg.

The power output of the cibarial pump dilators expressed as erg/sec/g of muscle and as g cm/sec/g of muscle are shown in Table 4. In C. lectularius the power developed by these muscles, like the muscular tension, decreases from the first to the second nymphal instar and then increases to a maximum in the adult stage. In the female A. aegypti the power output/g of muscle was higher than that of the first three nymphal instars and lower than that of the fifth instar and the adult of C. lectularius. It is also higher than that of the first two instars and lower than that of the adult P. humanus.

Roeder (1953) reported the power of the flight muscles in some insects. The minimum was 205 g cm/sec/g of muscle for Vanessa atalanta L. and the maximum was 558 g cm/sec/g of muscle for Aeschna mixta Latr. This indicates that the cibarial pump dilators in C. lectularius, A. aegypti, and P. humanus are not required to operate as high a specific power output as great as the flight muscles of the insects reported by Roeder (1953). The dilators of the cibarial pump of the fifth nymphal instar of R. prolixus, on the other hand, would, on the basis of Bennet-Clark data, be required to work at a greater specific power output than the flight muscles of these insects.

Table 4 - The power output of the cibarial pump dilators in C. lectularius, female A. aegypti, P. humanus, and R. prolixus.

Species	Travel of * diaphragm cm	Pump cycles/ second	Travel of diaphragm /sec cm	Power** Output erg/sec	Length of * muscles cm	Weight of muscles g	Power output	
							erg/sec/g	g cm/sec/g
C. lectularius								
First	2.4×10^{-3} (5) 2.1×10^{-3}-2.7×10^{-3}	4.0	9.6×10^{-3}	0.12	0.015 (6) 0.012-0.017	6.3×10^{-6}	1.9×10^{4}	19.4
Second	2.6×10^{-3} (7) 2.4×10^{-3}-2.8×10^{-3}	3.8	9.9×10^{-3}	0.11	0.017 (7) 0.013-0.019	8.7×10^{-6}	1.3×10^{4}	13.3
Third	3.6×10^{-3} (5) 3.2×10^{-3}-3.9×10^{-3}	3.3	1.2×10^{-2}	0.27	0.022 (5) 0.019-0.025	1.8×10^{-5}	1.5×10^{4}	15.2
Fourth	4.5×10^{-3} (5) 4.1×10^{-3}-4.9×10^{-3}	3.3	1.5×10^{-2}	1.20	0.026 (5) 0.021-0.031	3.1×10^{-5}	3.9×10^{4}	39.8
Fifth	6.2×10^{-3} (5) 5.8×10^{-3}-6.6×10^{-3}	3.0	1.9×10^{-2}	2.70	0.028 (8) 0.025-0.034	3.6×10^{-5}	7.5×10^{4}	76.5
Female	7.5×10^{-3} (8) 6.8×10^{-3}-7.9×10^{-3}	2.4	1.8×10^{-2}	4.30	0.033 (8) 0.029-0.037	6.9×10^{-5}	6.2×10^{4}	63.2
Male	7.5×10^{-3} (5) 6.8×10^{-3}-7.8×10^{-3}	2.4	1.8×10^{-2}	3.99	0.030 (5) 0.027-0.034	4.5×10^{-5}	8.9×10^{4}	90.8

Cont'd.

Table 4 - Continued

Species	Travel of diaphragm cm *	Pump cycles/ second	Travel of diaphragm /sec cm	Power output erg/sec **	Length of muscles cm *	Weight of muscles g	Power output	
							erg/sec/g	g cm/sec/g
A. aegypti Female	$3.3x10^{-3}$ (10) $2.9x10^{-3}$-$3.7x10^{-3}$	4.9	$1.6x10^{-2}$	0.09	0.013 (10) 0.010-0.015	$2.3x10^{-6}$	$3.9x10^{4}$	39.8
P. humanus								
First	$1.0x10^{-3}$-$3.9x10^{-3}$ (6) $9.5x10^{-4}$-$1.4x10^{-3}$	2.8	$3.0x10^{-3}$	0.003	$3.5x10^{-3}$ (6) $3.0x10^{-3}$-$3.9x10^{-3}$	$2.4x10^{-7}$	$1.3x10^{4}$	13.3
Second	$1.5x10^{-3}$ (5) $1.3x10^{-3}$-$1.7x10^{-3}$	5.3	$8.0x10^{-3}$	0.008	$5.2x10^{-3}$ (5) $4.9x10^{-3}$-5.5 x 10-3	$5.7x10^{-7}$	$1.4x10^{4}$	14.3
Female	$3.0x10^{-3}$ (7) $2.7x10^{-3}$-$3.4x10^{-3}$	4.6	$1.4x10^{-2}$	0.216	$9.2x10^{-3}$ (7) $8.8x10^{-3}$-9.5 x 10-3	$1.7x10^{-6}$	$1.3x10^{5}$	132.6
Male	$3.0x10^{-3}$ (6) $2.5x10^{-3}$-$3.4x10^{-3}$	4.2	$1.3x10^{-2}$	0.108	$8.7x10^{-3}$ (6) $8.3x10^{-3}$-9.0 x 10-3	$1.7x10^{-6}$	$6.3x10^{4}$	64.2
R. prolixus								
Fifth	0.016	3.0	0.048	912 $4.3x10^{3}$	0.05	$1.6x10^{-3}$	$5.7x10^{5}$ $2.7x10^{6}$	581.0 2752.3

* Average
(number of insects)
Range

** Power output = mean force x travel of the diaphragm in a second.

1.3.4. Discussion

The actual laws of blood flow, at least in the range of physiological rates of flow, approximate sufficiently the simple laws (e.g. Poiseuille's law) for this to be applied with caution, using the appropriate value for the effective viscosity rather than the obtained in viscometers of a large bore. Burton (1965) stated "As long as the diameter of the capillary tube used in the viscometer is more than 1 or 2 mm, the relative viscosity of the blood is the same, whatever the size of the tube used. When, however, tubes of narrower diameter are used, the value for relative viscosity found is less. This is because the absolute viscosity of water is the same however small the diameter of the tube; but that of the blood decreased to less than half the value found when large tubes are used." This has long been known as the Fahraeus-Lindqvist effect. From this the viscosity value and consequently the values of ΔP obtained in my calculations could be in error by a factor of 2. Burton (1965) also discusses the effect of temperature on viscosity of the blood. I should not expect significant variation in the viscosity of the blood by changes in temperature in the quick pumping process of sucking in these insects.

The ability to feed at a relatively high rate through a minute feeding canal is very important for blood-sucking insects. For this purpose they are equipped with very efficient feeding apparatus with a sucking pump which is capable of exerting a high tension on the blood upon which they feed. Differences in the rate of feeding in the different blood-sucking insects can be due to many factors. One of these factors is the negative pressure that can be produced in the cibarial pump. This in turn is dependent upon the tension which can be developed in the cibarial pump dilators, the length of the feeding canal and its diameter as well as the viscosity of the blood and the capillary blood pressure of the host. These experiments suggest that blood viscosity and the capillary blood pressure of the host might be of importance in the evolution of host selection in blood-sucking insects.

1.4. Effects of the size and the frequency of blood meals on *Cimex lectularius* L.

1.4.0. Methods

The threshold of hatching, nymphal development, and adult activity is between 13 and 15 C (Hase 1930, Mellanby 1935, Kemper 1936, Johnson 1942). The thermal death point is 44 C. Omori (1941) found that development ceased at 36 or 37 C. The temperature of 80 F (26.7 C) and 75% R.H. used for the stock culture proved to be in the range of optimum conditions for development, was used for the experiments. The insects were kept in 4 x 4 x 1.5 cm plastic boxes with folded pieces of filter paper and were allowed to feed twice every week on human blood. The insects fed through the organdie covering a 3 cm diameter hole in the lid of the plastic box. The eggs were laid on the folded filter papers which were collected in new boxes and the eggs were allowed to hatch at the same temperature and relative humidity.

Two series of experiments were conducted for studying the effects of different blood meal sizes on *C. lectularius*.

In the first series (1.4.1) newly moulted insects, taken from the standard culture, were kept singly in 2 x 7 cm specimen tubes with a 2 cm^2 piece of folded filter paper. The insects were fed only once on the second day after moulting. Feeding periods of five or six different lengths were used for each instar to provide the different meal sizes. For each feeding period 20 insects were used from every instar. Observations

on subsequent moulting and longevity were recorded daily.

The efficiency of the various instars in converting human blood into body tissue and extra-cellular fluid was determined by the method given by Friend et al. (1965). The amount of blood required to produce 1 mg gain in body weight was calculated. The average difference in body weight between instars divided by the weight of the blood meal and multiplied by 100 is the food conversion efficiency%(Friend et al. 1965). Twenty first instar nymphs were taken from the standard culture immediately after hatching for determining the weight changes during development and the food conversion efficiency of the different instars. These insects were fed to capacity on the second day after hatching and once every ten days at least thereafter. The changes in body weight were recorded during development till the insects reached maturity. This way of estimating food conversion efficiency is subject to certain errors. Calculation of food conversion efficiency is always done on dry weight basis, but it will be also subject to some errors. The most important of these arise from changes and differences in water content of both blood and insects, and from the presence of blood residues in the gut.

Newly moulted adults were used for studying the effects of different blood meal sizes on the duration of the preoviposition period, fecundity and longevity. The following combinations of males and females and blood meal size were studied:

unfed female + engorged male
female fed for 60 seconds + engorged male
female fed for 120 seconds + engorged male
female fed for 240 seconds + engorged male
engorged female + unfed male
engorged female + engorged male
engorged female + male fed for 60 seconds
engorged female + male fed for 120 seconds
engorged female + male fed for 240 seconds.

For each combination 20 pairs were used and each pair was put in a single 2 x 7 cm specimen tube together with a 2 cm^2 piece of filter paper.

In the second series of experiments (1.4.2) eggs were taken from the standard culture and put separately in 2 x 7 cm specimen tubes each with a piece of filter paper. The insects were fed on the second day after hatching. Eighty insects in four groups of 20 were used for the study of the effects of each feeding period. The four groups were given the blood meal of known duration at frequencies of 2,4,8, and 16 days respectively. Observations were carried out daily and the effects of the different feeding periods and their frequencies on the duration of the nymphal stadia, and on the preoviposition period; fecundity, longevity and weight changes during development were recorded.

As most of the insects did not reach the adult stage when the feeding periods were 15,60 or 120 seconds, at the different

frequencies, an additional experiment was conducted. One hundred and sixty pairs in four groups of forty were taken from the standard culture as soon as they moulted from the fifth instar and used to complete the study of the effects of the size and frequency of blood meals on the duration of the preoviposition period, fecundity and longevity of the female and male. The feeding periods used were 15,30,60, and 120 seconds at the same frequencies as before. Ten pairs were used for each frequency.

1.4.1. Effects of the size of one blood meal per instar

1.4.1.0. On the nymphal instars

The effects of the size of one blood meal per instar on percent moulted, duration of the nymphal stadia, longevity and mortality rates were studied. Statistical analysis was undertaken to determine the level of correlation between the amount of blood ingested and each of duration of the nymphal stadia and longevity. The correlation coefficient r was calculated from the formula:

$$r = \frac{\Sigma xy - \frac{(\Sigma x)\ (\Sigma y)}{n}}{\sqrt{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}}$$

where x = the average weight of the blood meal

y = the mean duration of the nymphal stadium or the mean longevity.

A theoretical straight line relationship was assumed for the effect on the duration of the nymphal stadia and the longevity. The average decrement in the duration of the nymphal stadium and the average increment in the longevity per unit increase in the weight of the blood meal were estimated by calculating the coefficient of linear regression b from the formula:

$$b = \frac{\Sigma xy - \frac{(\Sigma x)\ (\Sigma y)}{n}}{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]}$$

The discrepancies between the observed values and the theoretical

ones were shown by calculating the chi square to test the goodness of fit to the straight lines.

The effect of the size of one blood meal per instar on the percentage of nymphs which moulted in the different instars is shown in Figures 6a to 6e and Tables 12a to 12e of the appendix. No insect in any instar moulted if fed for 30 seconds or less. In all instars 100% moulting occurred if the insects were fed till engorgement. In all the five nymphal instars there were positive correlations between the size of the blood meal and the percentage of nymphs which moulted as the correlation coefficient ranged between 0.916 and 0.999.

The minimum feeding period for a single blood meal to induce moulting was 60 seconds in the first and second instars, 40 seconds in the third instar, and 120 seconds in both the fourth and fifth instars. These periods are all less than half of the maximum feeding periods. Friend et al. (1965) stated that *R. prolixus* nymphs of all instars will moult normally when fed less than 50% of the maximum meal and for the third instar only 24.7% of the maximum was required. Locke (1958) caused fourth-instar nymphs of *R. prolixus* to moult by feeding them 35% of the maximum meal. The blood meal supplies the nutrients and stretches the abdomen. The latter initiates the hormone cycle that results in moulting (Friend et al. 1965). W~~r~~igglesworth (1963) demonstrated that nutrients

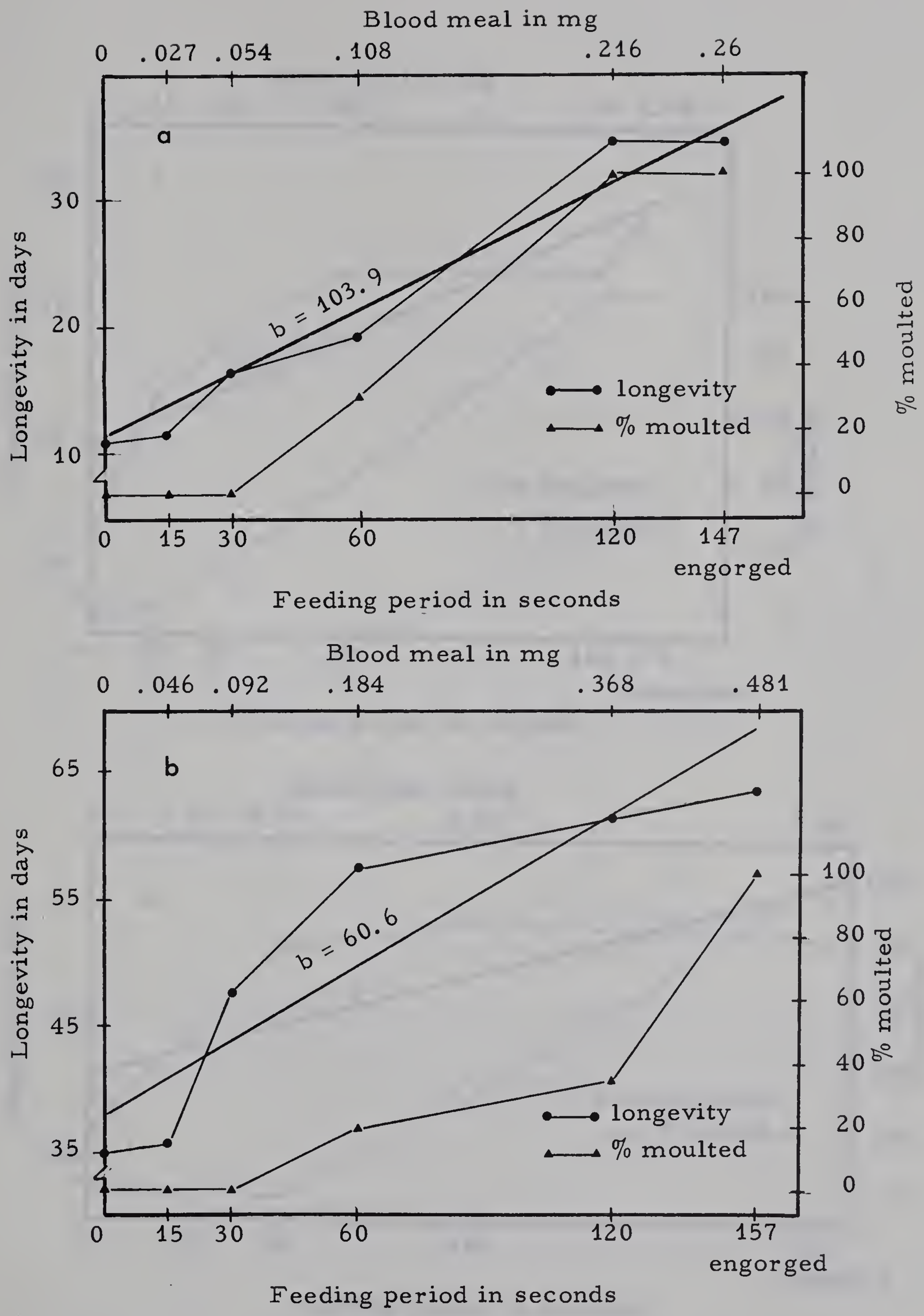


Fig. 6. Effect of the size of a single blood meal on the longevity and moulting of nymphs of C. lectularius. a. first instar; b. second instar

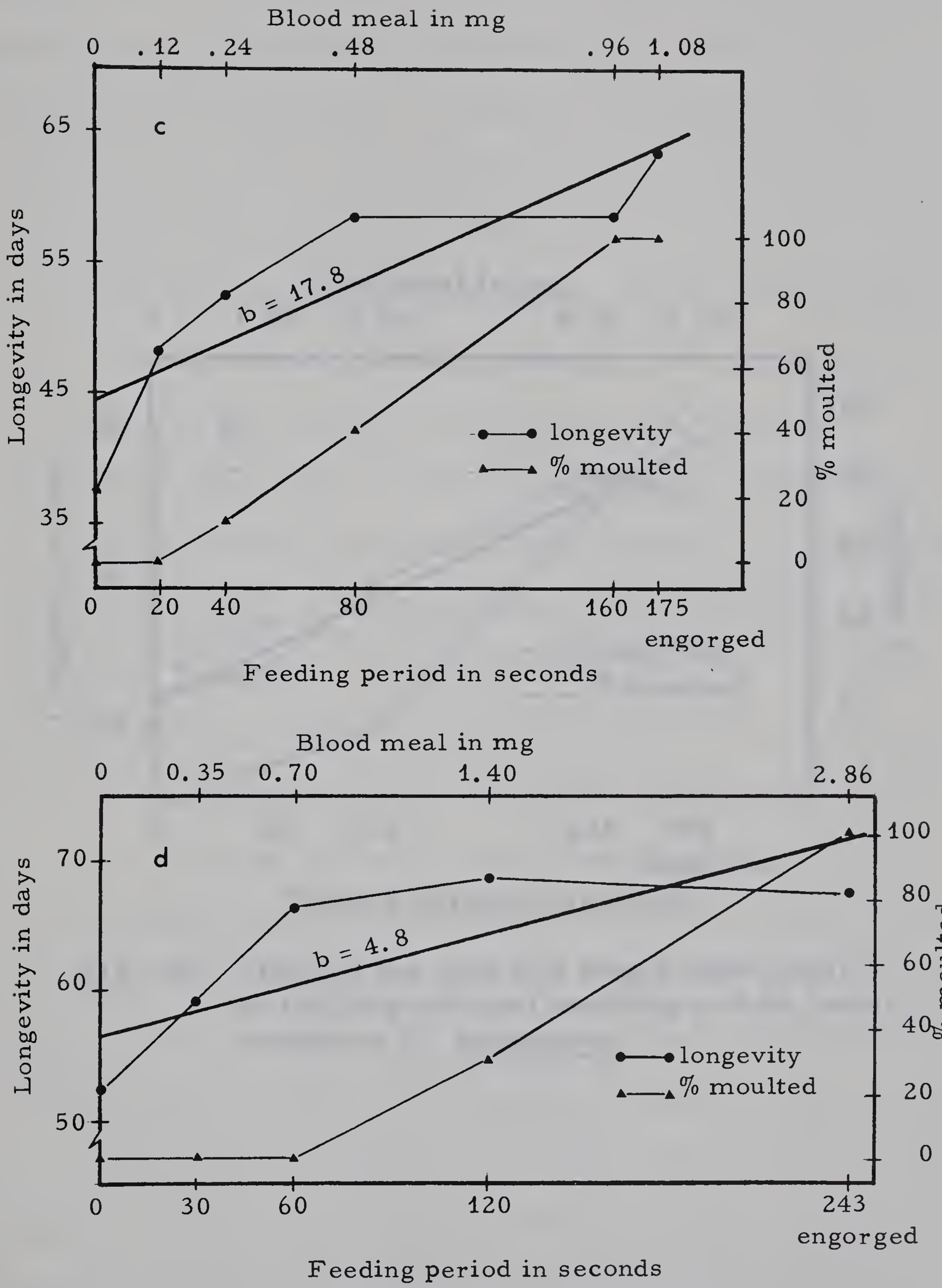


Fig. 6. - "Continued." c. Third instar; d. Fourth instar.

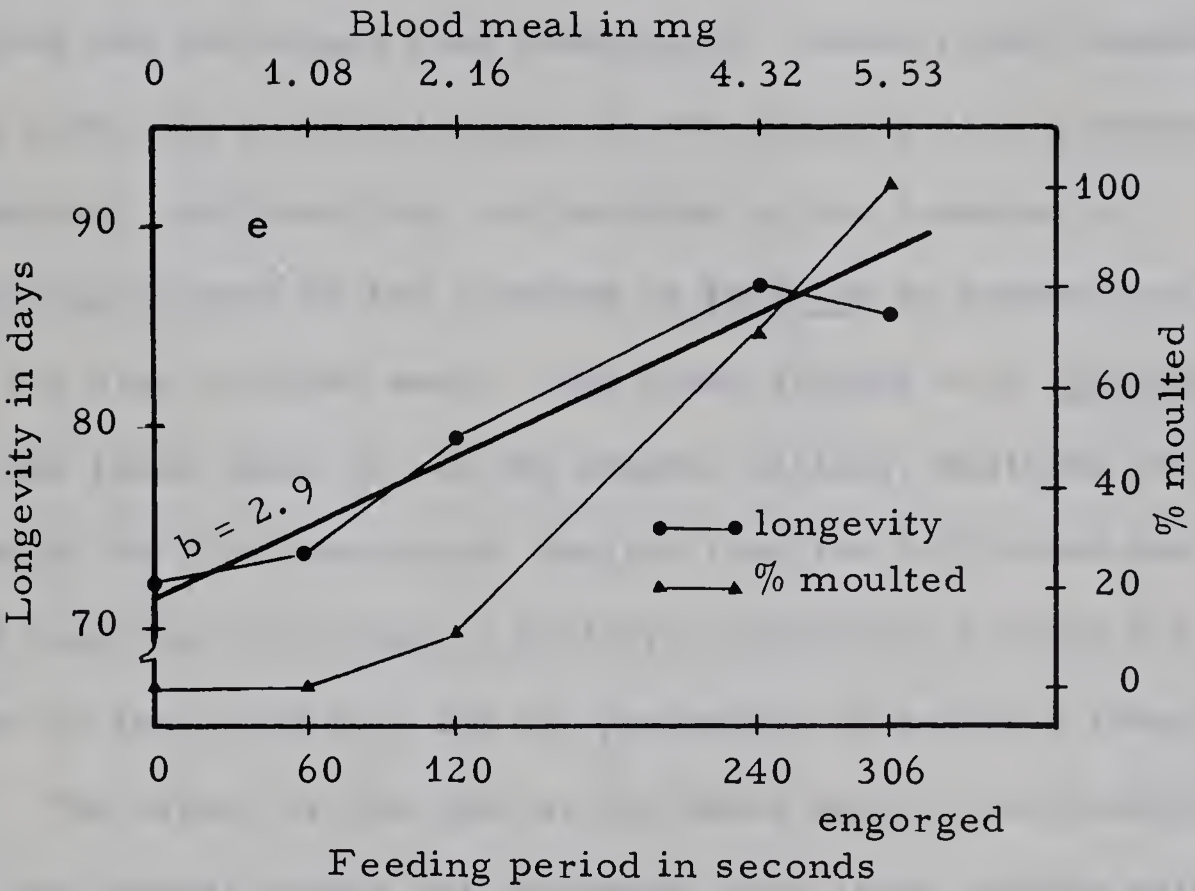


Fig. 6e. Effect of the size of a single blood meal on the longevity and moulting of fifth instar nymphs of C. lectularius.

alone do not stimulate moulting and this was supported by the work of Beckel and Friend (1964). The latter workers explained that release of the moulting hormone, and activation and division of the epidermis is caused by stretching and they claimed that in their experiments, because of either inadequate nutrient or some undiscovered factor, the moulting cycle was halted and the animal died prematurely. Locke (1958) studied the effect of the blood intake on the diameter of the tracheae produced. He found that the increase in the diameter at moulting in many of the tracheae in *Rhodnius* is proportional to the size of blood meal. From these studies on *C. lectularius* it was found that, in all the nymphal instars, moulting can be induced by blood meals much smaller than the full blood meal and that there is always a positive correlation between the size of the blood meal and the percentage of moulting insects.

The effect of the size of the blood meal on the duration of the nymphal stadia was determined from those insects which moulted, in each group. The duration of the nymphal stadium was taken as the period elapsing between the moult before feeding and that after feeding. The results are shown in Table 5. In all the five nymphal instars there was a slight decrease in the duration of the nymphal stadium with the increase in the size of the blood meal. The correlation coefficient ranged between -0.79 and -0.99.

Figures 6a to 6e and Tables 12a to 12e show the effect of the size of the blood meal on the longevity of the different

Table 5 - Effect of the size of a single blood meal on the duration of the different stadia of C. lectularius.

Feeding period in seconds	First stadium		Second stadium		Third stadium		Fourth stadium		Fifth stadium	
	x*	y**	x	y	x	y	x	y	x	y
Unfed	0	-	0	-	0	-	0	-	0	-
15	0.021	-	0.046	-						
20					0.124	-				
30	0.041	-	0.092	-			0.035	-		
40					0.248	7 (2) 7				
60	0.083	5 (6) 5	0.184	7 (7) 7			0.704	-	1.086	-
80					0.496	7 (8) 7				
120	0.165	4.7±0.15 (20) 4 - 5	0.368	7 (7) 7			1.408	7 (6) 7	2.172	8 (2) 8
160					0.992	6.4±0.01 (20) 6 - 7				
240									4.345	6.2±0.08 (14) 6 - 7
Engorged	0.260	4.5±0.13 (20) 4 - 5	0.481	6.3±0.01 (20) 6 - 7	0.087	5.6±0.01 (20) 5 - 6	2.856	6.6±0.01 (20) 6 - 7	5.533	6 (20) 6
Correlation coefficient r between x and y	-0.97		-0.79		-0.89		-0.99		-0.96	
Regression coefficient b	-2.77		-2.13		-1.5		-0.28		-0.63	

* x = average weight of the blood meal in mg.

** y = mean duration of the stadium ± S.E.
(number of insects)
Range

instars. The longevity of all five instars increased gradually with the increase in the size of the blood meal. The correlation coefficients ranged from 0.773 to 0.993. In the first four instars chi square tests showed departure from a straight line relationship. In the fifth instar, on the other hand, the relationship proved to be a straight line.

To estimate the effect of the size of a single blood meal on the mortality rate of the nymphal instars analysis of the data was undertaken using the approximate method of probit analysis (Finney 1947). The L.T. 50, which is the time at which 50% of the nymphs had died, was found for each blood meal size from the provisional probit line (Figures 7a to 7e). The reliability of the estimate is such that if the experiment is repeated there is 5% chance of getting an L.T. 50 value that is not within the fiducial limits.

The L.T. 50 values and their fiducial limits are shown in Table 6. In all the five nymphal instars it was found that the L.T. 50 increases with the increase in the size of the blood meal.

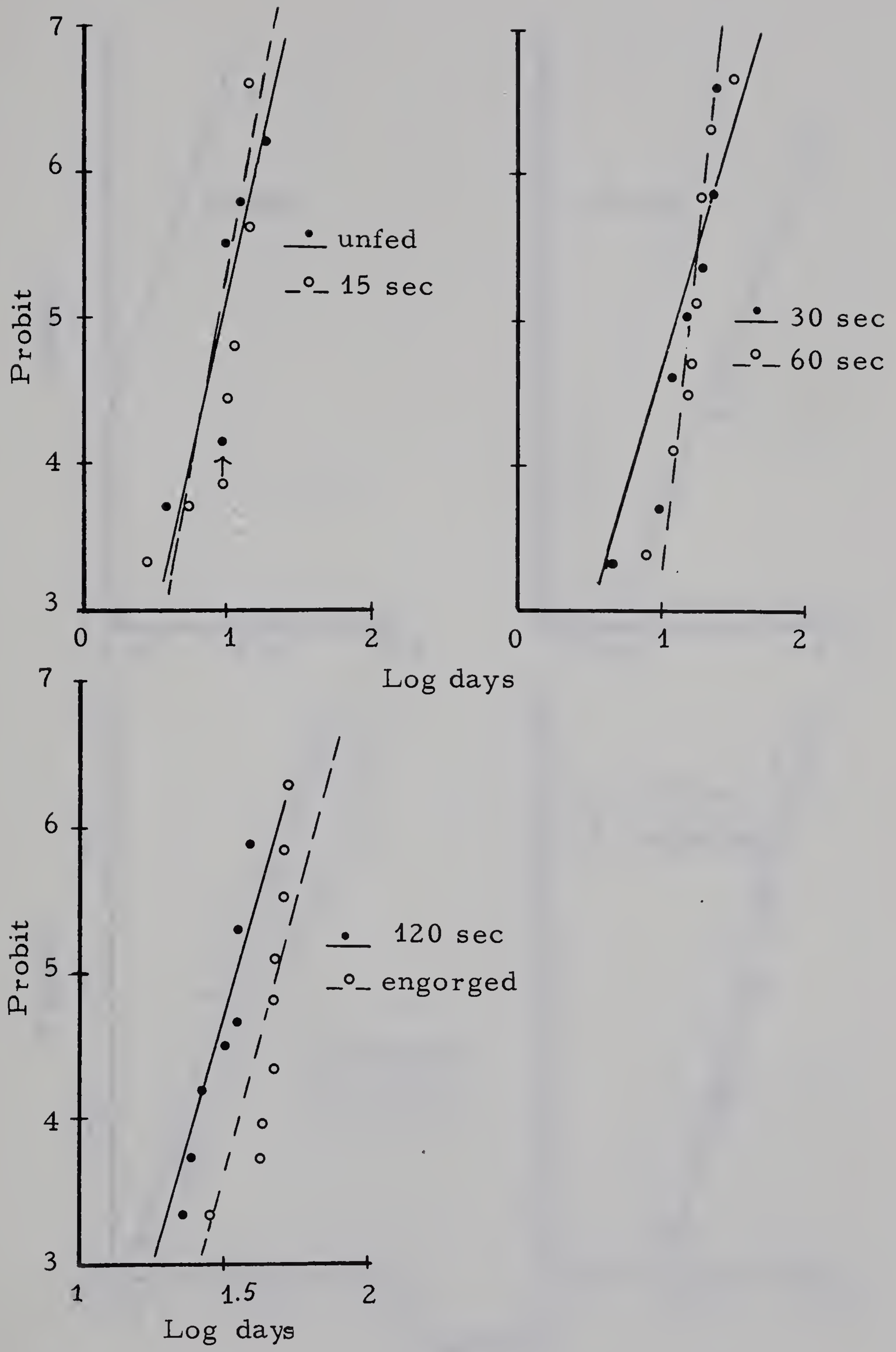
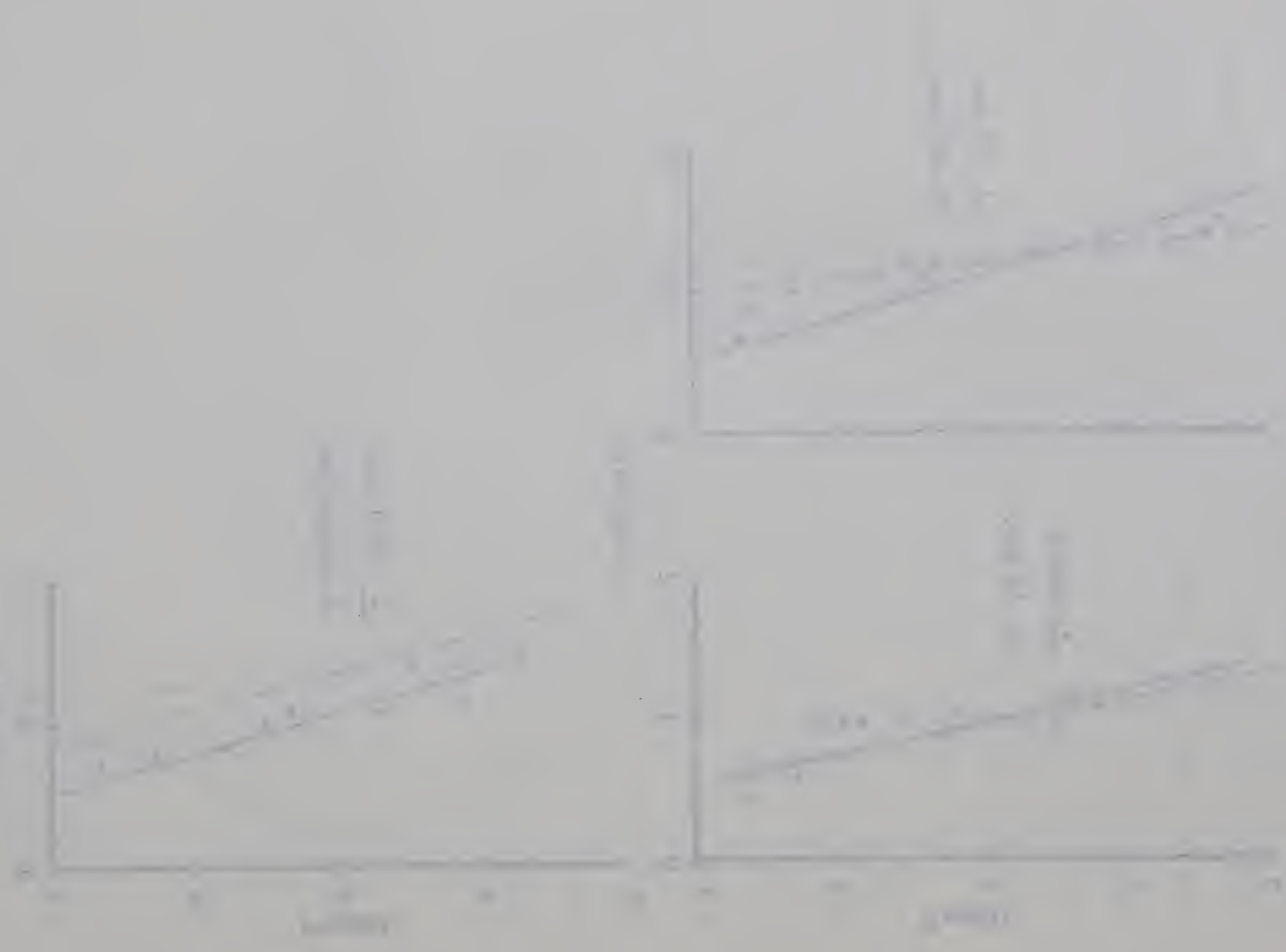


Fig. 7a. Probit lines for first instar of C. lectularius.



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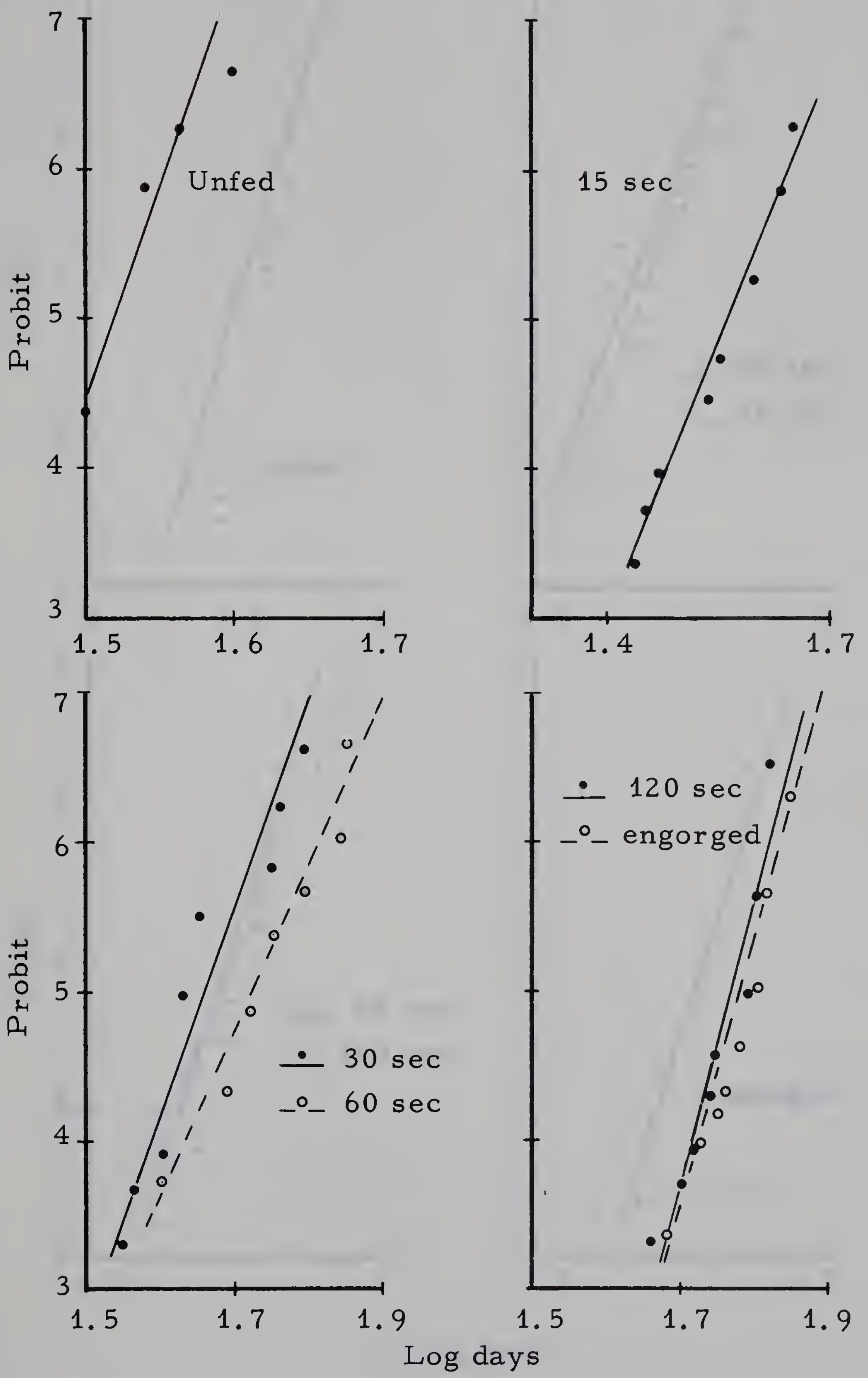

Fig. 7b. Probit lines for second instar of C. lectularius.

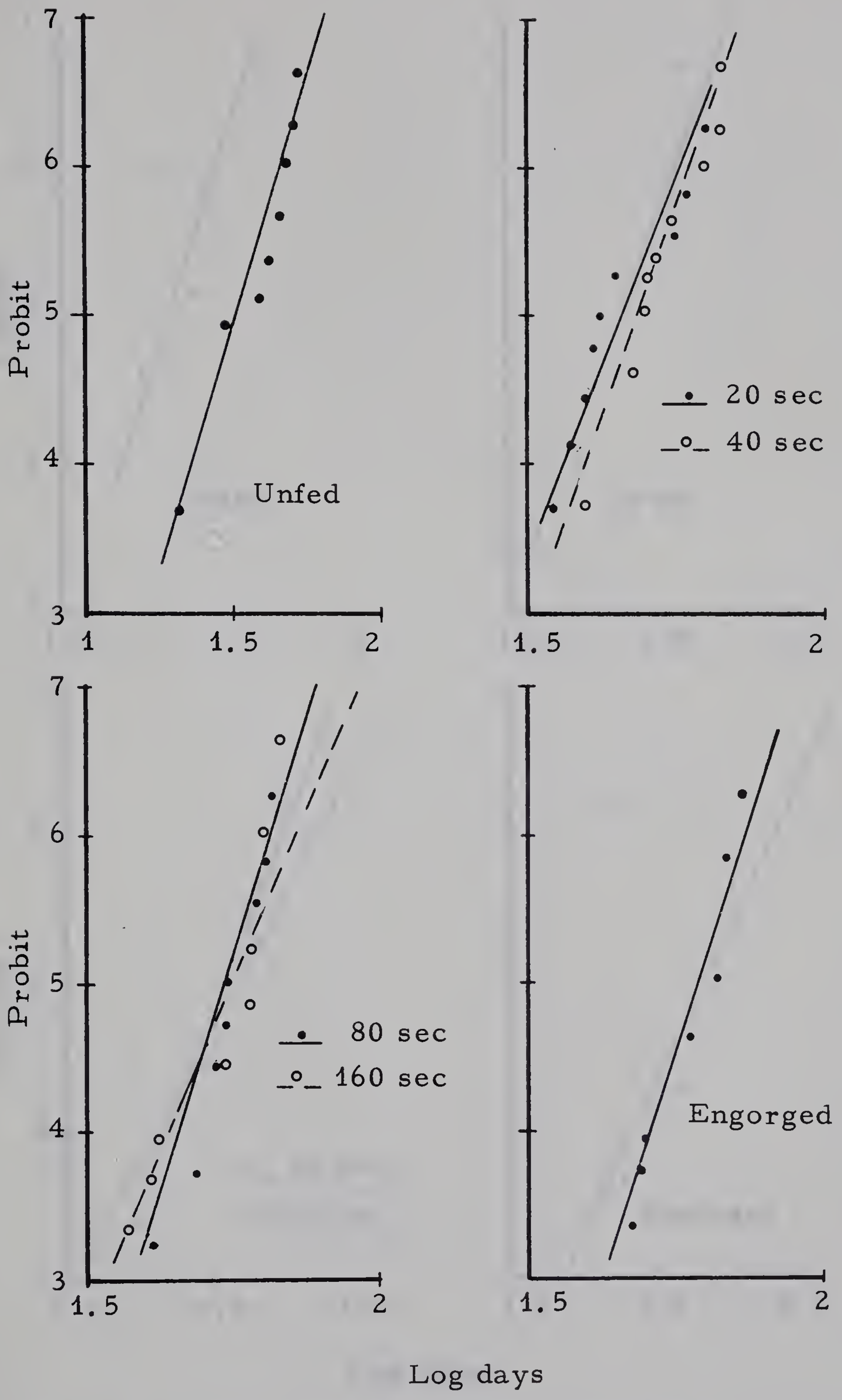

Fig. 7c. Probit lines for third instar of C. lectularius.

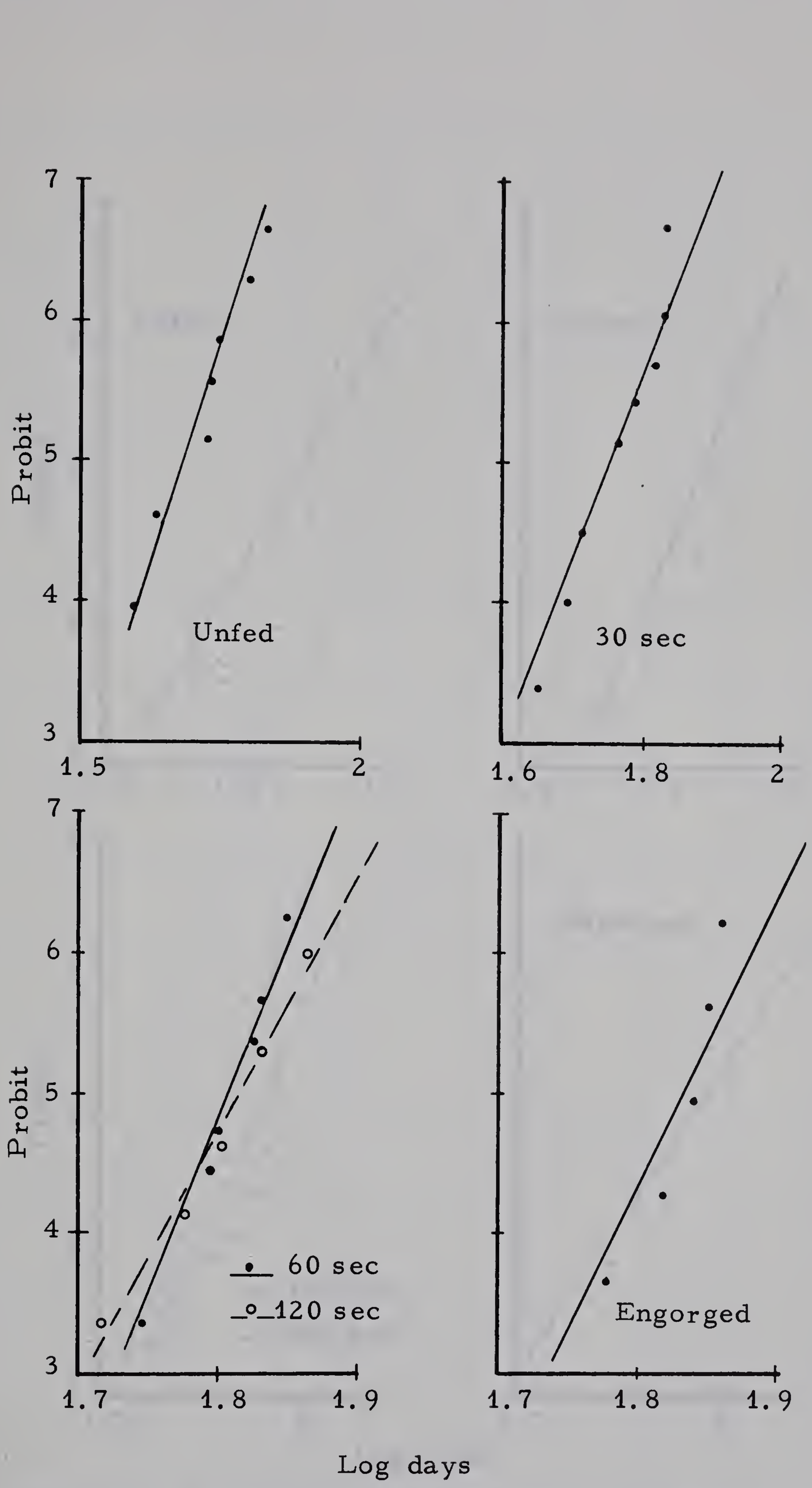

Fig. 7d. Probit lines for fourth instar of C. lectularius.

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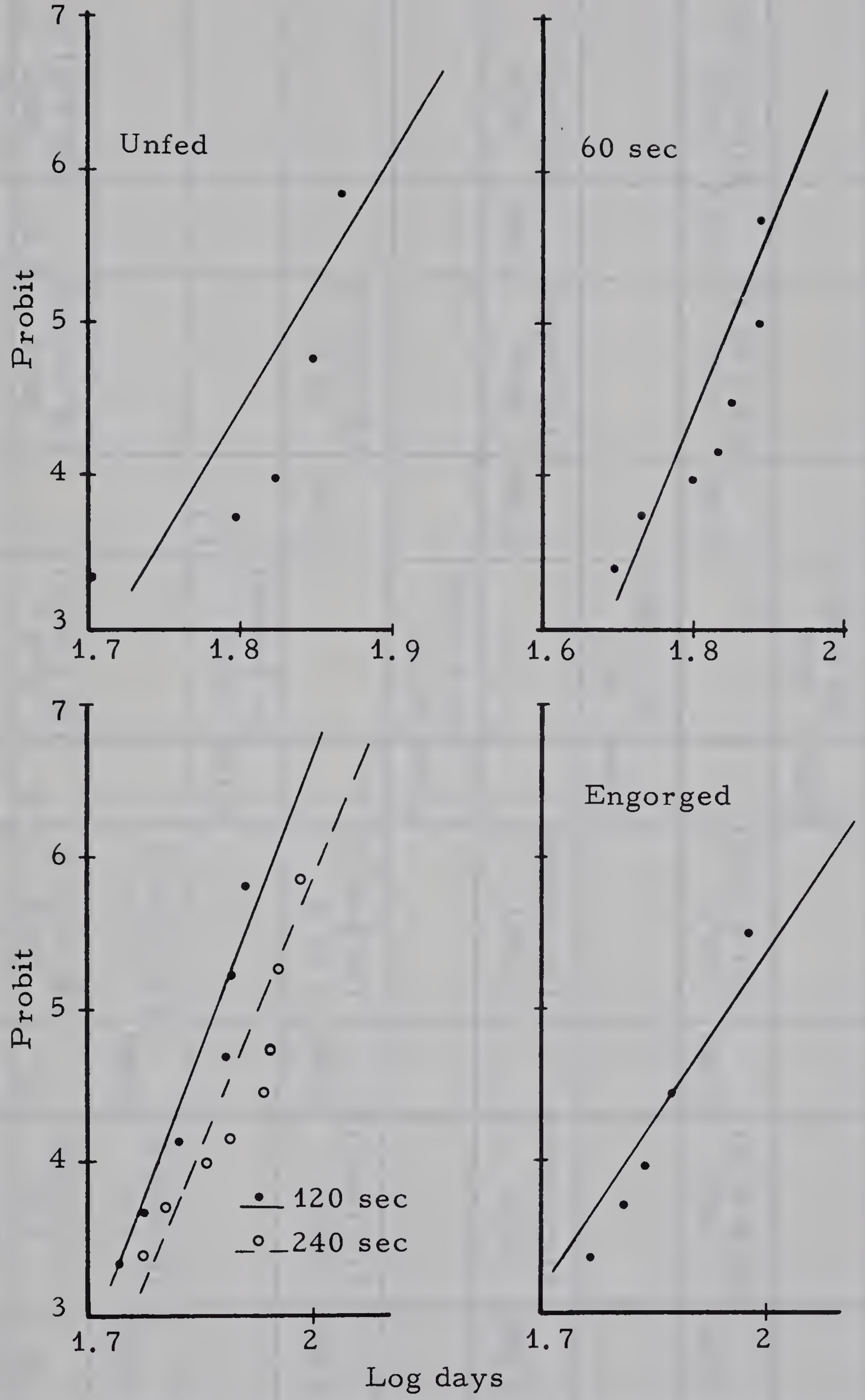

Fig. 7e. Probit lines for fifth instar of C. lectularius.

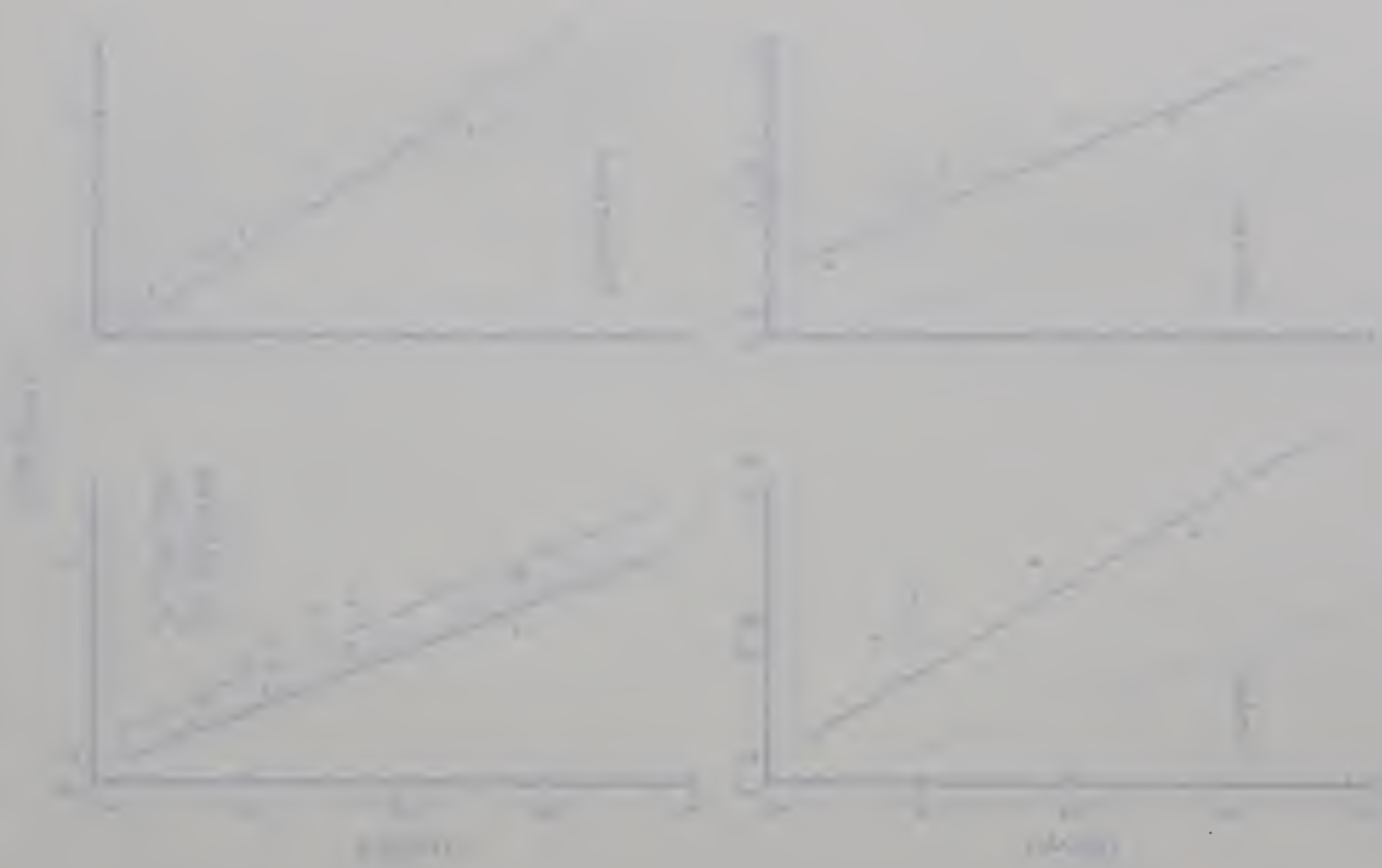

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Table 6 - Effect of the size of a single blood meal on the L.T. 50 in the different instars of *C. lectularius*.

Feeding period in seconds	First instar		Second instar		Third instar		Fourth instar		Fifth instar	
	x̄*	y**	x	y	x	y	x	y	x̄	y
Unfed	0	9.0 (7.5-10.8)	0	33.3 (32.2-34.3)	0	31.6 (28.7-35.1)	0	48.4 (46.2-50.8)	0	68.7 (66.4-71.1)
15	1.021	9.3 (8.3-10.5)	0.046	37.2 (35.4-39.1)						
20					0.124	44.7 (42.6-46.8)				
30	0.042	12.6 (10.7-14.7)	0.092	45.9 (44.2-47.7)			0.352	58.9 (57.1-60.7)		
40					0.248	47.9 (45.8-50.0)				
60	0.083	15.5 (14.6-16.4)		53.1 (50.3-56.0)			0.704	64.4 (63.0-66.0)	1.086	70.8 (68.0-73.7)
80					0.496	53.7 (51.5-56.0)				
120	0.165	36.3 (33.8-39.0)	0.368	58.9 (57.2-60.6)			1.408	65.3 (63.1-67.7)	2.172	74.5 (71.2-77.9)
160					0.992	56.2 (53.3-59.3)				
240									4.345	83.6 (80.3-87.0)
Engorged	0.260	47.9 (46.7-49.1)	0.481	66.1 (63.8-68.4)	1.087	61.0 (58.4-63.6)	2.856	67.6 (65.9-69.4)	5.533	89.1 (80.9-98.2)

* x = average weight of the blood meal in mg.

** y = L.T. 50
(95% confidence limit)

Figure 8 shows the average weight changes during development of the nymphal instars when they take a full blood meal in each instar. The sudden rise in weight after taking the blood meal is followed by a rapid fall which is mostly due to the passage of the water and some other constituents of the blood during the first day after the meal. Over the period preceeding the next meal the weight keeps dropping at a progressively slower rate. Titschack (1930) published a similar curve with small differences in weights. Przibram has proposed that the weight of an insect is doubled at each instar and the linear dimensions increased by 1.26 at each moult (Wigglesworth 1965). Although this rule is more applicable to hemimetabola than to holometabola, it is of doubtful value for C. lectularius because its growth curve appears discontinuous due to the ingestion of blood meals 3 to 5 times the body weight.

Table 7 shows that the food conversion efficiency in the different nymphal instars of C. lectularius varies between 25.6 and 37.0%. It takes about 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. The third instar is less efficient than are the others. Comparison of Table 7 with the results of Jones (1930) shows a similarity in the trend of the food conversion efficiency in the different instars with small differences in the values. The values shown in Johnson's Table III (1960) are all higher than mine because he weighed

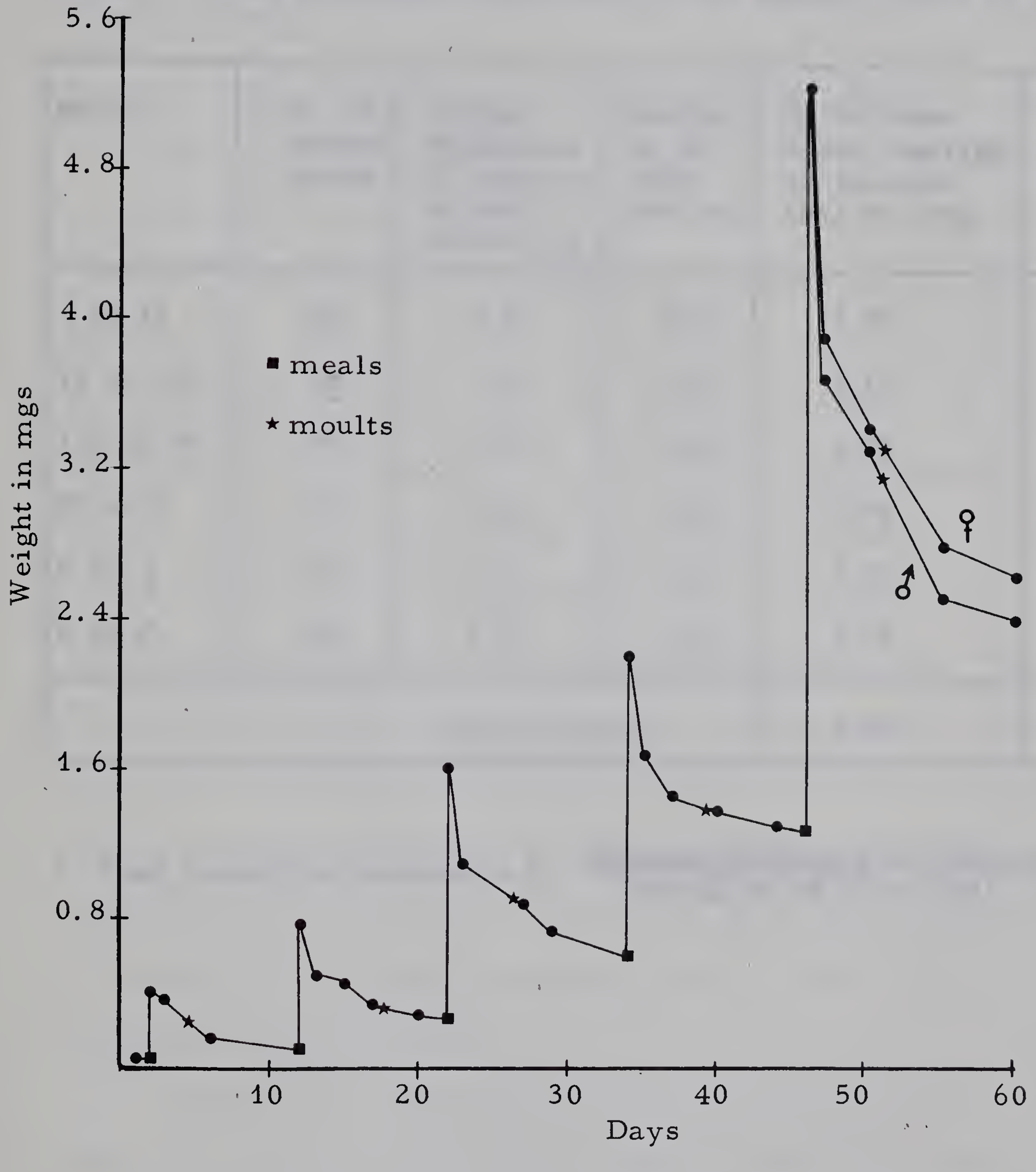

Fig. 8. Weight changes during development of C. lectularius.

Table 7 - Food conversion efficiency in the nymphal instar of *C. lectularius*.

Moult	No. of insects tested	Average difference in body wt between instars,mg	Average wt of blood meal,mg	Mg of human blood required to increase body wt 1 mg	Food* conversion efficiency %
I to II	20	0.07	0.25	3.57	28.0
II to III	20	0.20	0.63	3.15	31.8
III to IV	20	0.27	1.02	3.90	25.6
IV to V	20	0.67	1.81	2.70	37.0
V to ♀	20	1.37	4.18	3.05	32.8
V to ♂	20	1.12	4.18	3.73	26.8
Overall means				3.70	30.3

* Food conversion efficiency % = $\frac{\text{Average difference in body wt}}{\text{Average wt of blood meal}} \times 100$

the nymphs a short period after feeding. There is a difference in food conversion efficiency between the fifth instar and the adult male and female. This was overlooked by Jones (1930) and Johnson (1960).

1.4.1.1. On the duration of the preoviposition period

The effect of the size of the blood meal on the duration of the preoviposition period is shown in Figures 9A and 9B and Table 13. In the first five experiments, Figure 9A, the males were fed till engorgement and the females for different periods. No oviposition took place when the females were unfed. It was observed that an increase in size of the female's blood meal caused a gradual decrease in the preoviposition period.

A summary of the statistical analysis concerning the first five experiments in Table 13 is shown in Table 14. The value of the correlation coefficient r (-0.908) indicates that there is a high negative correlation between the size of the female's blood meal and the preoviposition period when the mating males were fed till engorgement. The highly significant value for chi square shows departure from the theoretical straight line relationship.

In Figure 9B, experiments 5 to 9 Table 13, the females were fed till engorgement and the males for different periods. There was no significant difference in the duration of the preoviposition period.

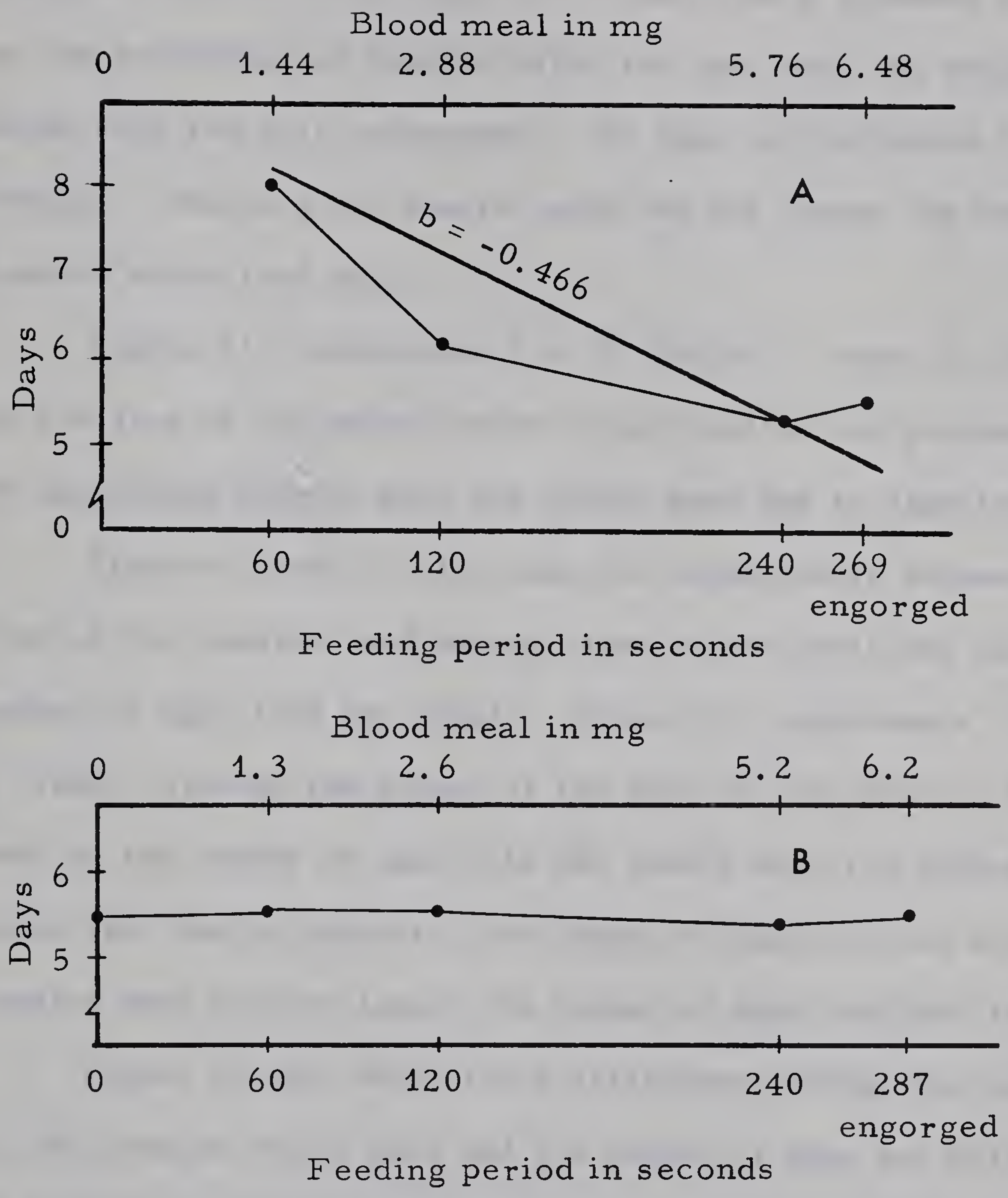

Fig. 9A. Effect of the size of the female's blood meal on preoviposition period of C. lectularius.

9B. Effect of the size of the mating male's blood meal on the preoviposition period of engorged female C. lectularius.

1.4.1.2. On fecundity

From Figure 10, experiments 1 to 5 Table 13, it is clear that the size of the female's blood meal has a profound effect on the percentage of females which lay eggs when the mating males were fed till engorgement. No eggs were produced by unfed females. The more the females were fed the larger the number of females which laid eggs.

Figure 11, experiments 5 to 9 Table 13, shows no effect of the size of the mating males' blood meal on the percentage of egg laying females when the latter were fed to capacity.

Figures 10 and 11 also show the relationship between the size of the females' and mating males' blood meals and the number of eggs laid per female. Figure 10, experiments 1 to 5 Table 13, shows the effect of the size of the females' blood meal on the number of eggs laid per female when the mating males were fed to capacity. As shown in Figure 10 the more the females were fed the larger the number of eggs laid per female.

Figure 10 also shows the relationship between the size of the females' blood meal and the number of eggs per milligram of blood (the first five experiments Table 13). From this figure it is clear that there is a slight increase in the number of eggs laid per milligram of blood with the increase in the size of the female's blood meal within the range of experiments 2 to 4. On the other hand, there is a high increase from experiment 4 to 5.

Egg laying in C. lectularius has been studied under various conditions (Hase 1930, Titschack 1930, Omori 1941, Johnson 1942).

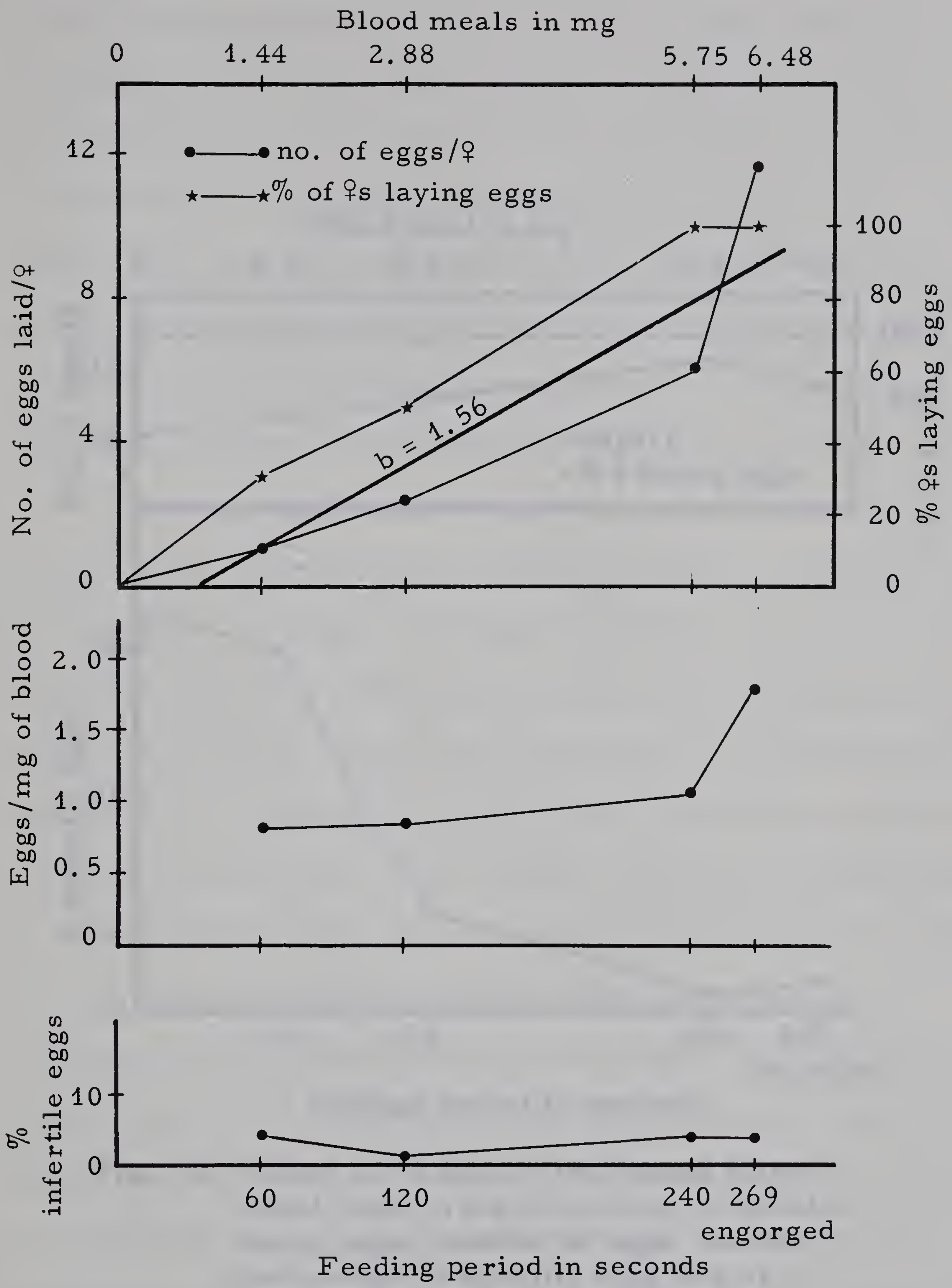

Fig. 10. Effect of the size of the blood meal of female *C. lectularius* on the percentage laying eggs, number of eggs laid per female, number of eggs laid per mg of blood, and percentage of infertile eggs.

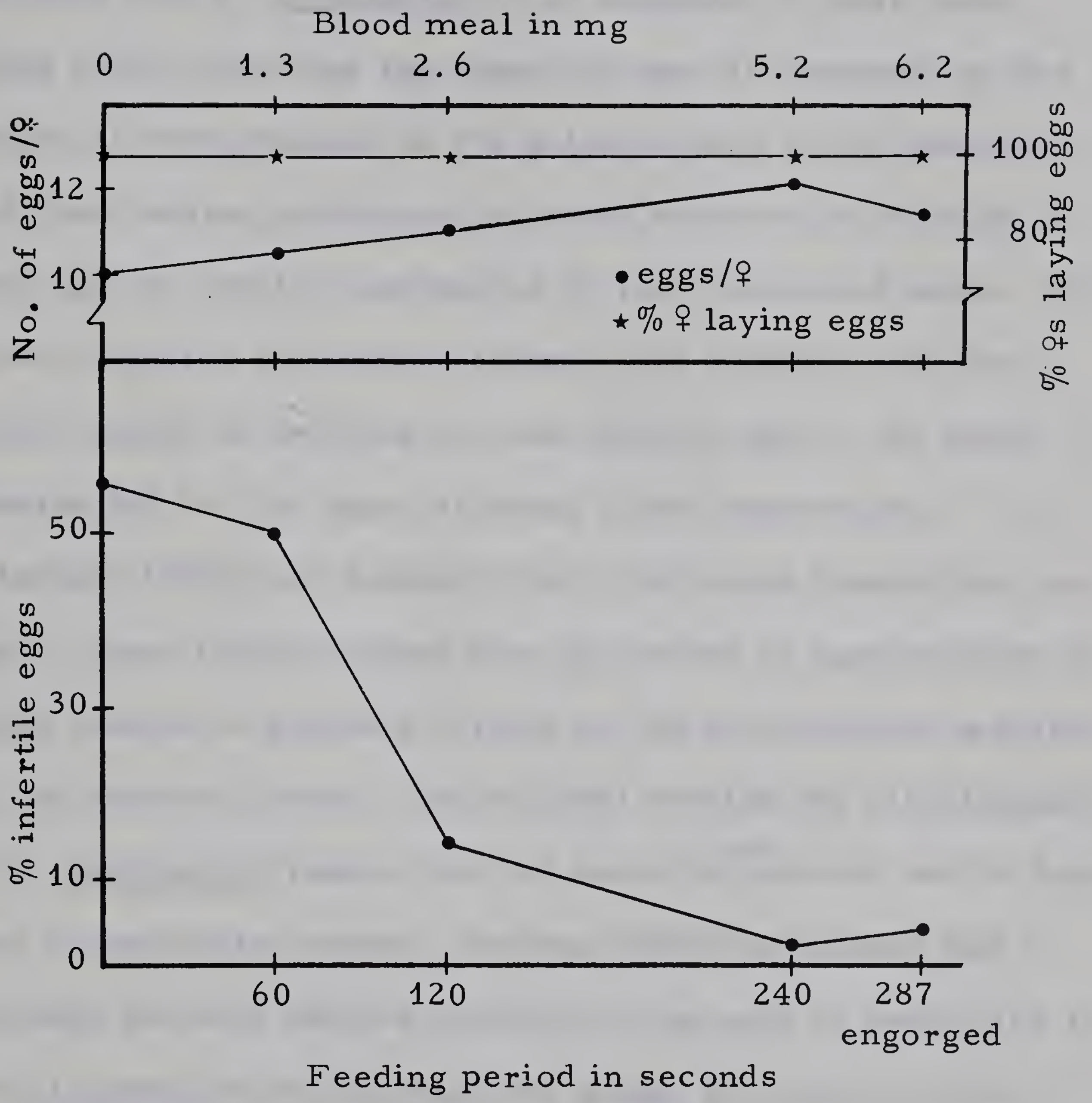

Fig. 11. Effect of the size of the mating males' blood meal on the percentage of females laying eggs, number of eggs, and the percentage of infertile eggs laid by engorged female C. lectularius.

In most blood-sucking insects, the number of eggs produced by a female is correlated, within limits, with the quantity of food taken (Wigglesworth 1960). The results shown in Figure 10 indicate that *C. lectularius* is no exception to this rule. Cragg (1923) said that the number of eggs is dependent on the amount of food obtained by the males as well as the females, and that females impregnated by unfed males do not produce as many eggs as females impregnated by fully nourished males. My studies confirm the former statement but indicate that the latter should be modified to read "fertile eggs". My unfed females did not lay eggs, although it was reported by Titschack (1930) and Johnson (1942) that unfed females can lay eggs. Jones (1930) claimed that the number of eggs produced by unfed females is probably related to the food reserves acquired in the previous instar. Davis (1964) studied the vitellogenesis of *C. lectularius* female that had mated but were starved and he found that no maturation occurs. Gooding (1963) postulated that although the most obvious function of the meal in human lice is that it provides the material and energy for growth of the nymphs and the maturation of eggs in the adult stage, it may also function as a stimulant resulting in the formation and/or release of hormones. Johansson (1964) said "after an adequate meal the "nervous?" stimuli from the gut activate the corpus allatum by way of the brain. Hormones from the corpus allatum and the neurosecretory cells in the brain stimulate the ovaries and accessory glands. In the absence of adequate food the brain

inhibits the corpus allatum and the hormone titre remains too low for the ovaries and accessory glands to function normally." In C. lectularius it seems that there are more subtle factors involved in the production of eggs than mere quantities of blood taken. Bell and Schaefer (1966) found that the highest average for egg production in C. lectularius (5.4) was by those fed on 9:1 mixture of whole rabbit blood and insect Ringer's, and the minimum was with females fed on Ringer's alone.

Figures 10 and 11 and Table 13 show the effect of each experiment on the percentage of infertile eggs laid. Figure 10, experiments 2 to 5, shows that the size of the female's blood meal has no significant effect on the percentage of infertile eggs laid. On the other hand, Figure 11, experiments 5 to 9, shows that the size of the blood meal of the mating male has a significant effect on the percentage of infertile eggs laid by the females.

Titschack (1930) found that the percentage of sterile eggs increased from 0 to 1% during the fertile period of C. lectularius. In 1939a Mellanby found that mating is a necessary process for egg production. Davis (1965b) studied the effect of insemination in activating the corpus allatum of female C. lectularius and he found that the mechanical aspects of insemination play no role in this activation and concluded that the presence of spermatozoa in the conceptacula or lateral oviducts stimulates the corpus allatum. He found that 3 to 4 hours are required for seminal stimulus to become effective and this

period corresponds with the time required for the spermatozoa to reach the genital tract. He also suggested that the seminal stimulus results from summation of stimuli of many receptors on the genital tract.

1.4.1.3. On longevity of the adult

The effect of the size of the blood meal on the longevity of the adult female is shown in Figure 12 and Table 15. Analysis of the data was undertaken on the basis of dividing the results into longevity of females which did not lay eggs, longevity of the females which laid eggs and longevity of both. In all the three cases there was a positive correlation between the size of the blood meal and the longevity of the females (Table 15). A straight line relationship was found between the size of the blood meal and longevity of the females which did not lay eggs. Similarly, there was a straight line relationship between the size of the blood meal and the longevity of all the females. On the contrary, the straight line relationship did not hold for females which laid eggs alone. This indicates that when the females take smaller blood meals, egg production may have an influence on their longevity.

Figure 12 and Table 15 also show the relationship between the size of the blood meal and the longevity of males. It was observed that the males lived longer when the blood meal was increased in size and that the relationship was a straight line.

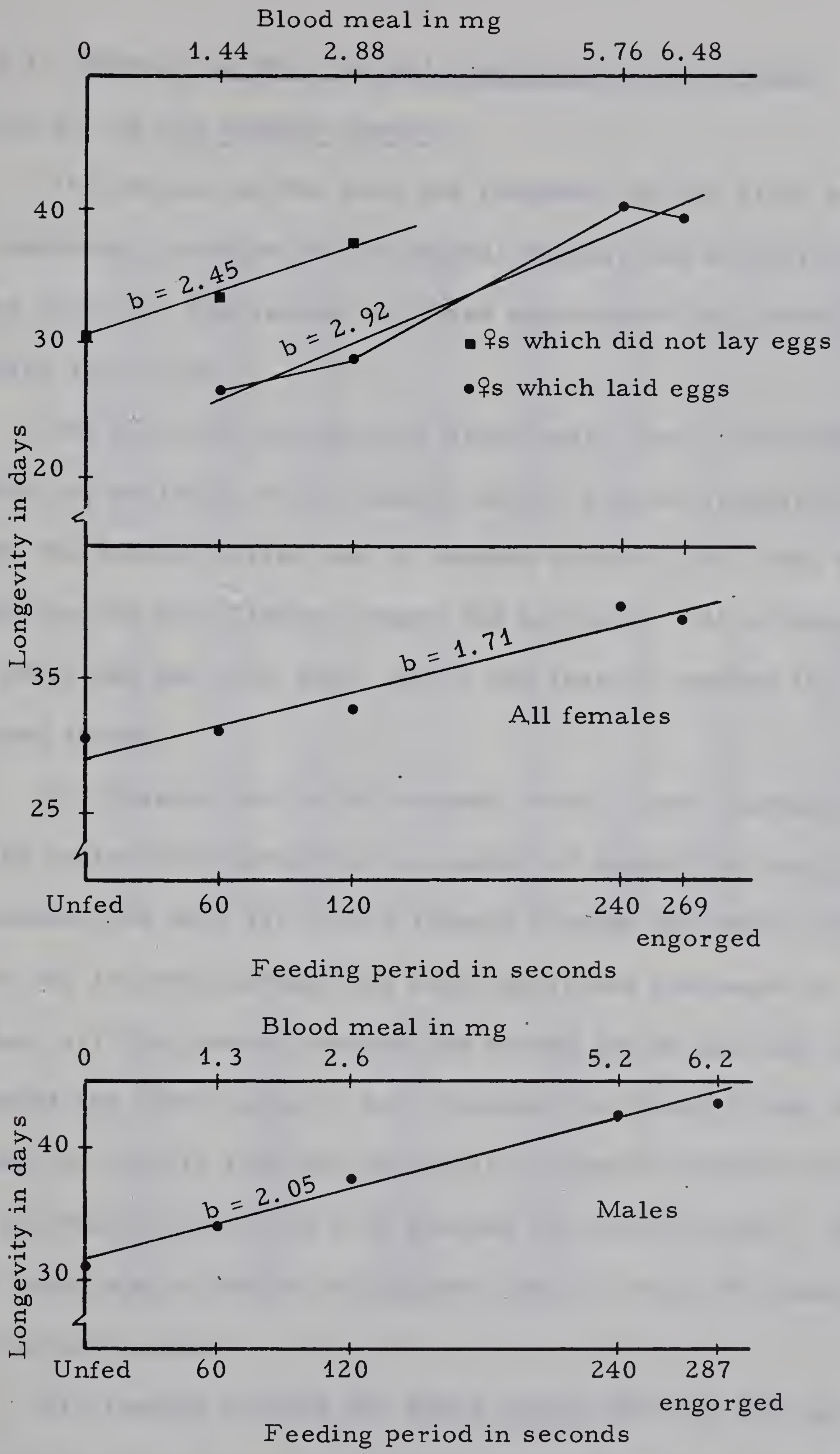

Fig. 12. Effect of the size of a single blood meal on the longevity of the female and male C. lectularius.

1.4.2. Effects of the size and frequency of blood meals.

1.4.2.0. On the nymphal instars

The effects of the size and frequency of the blood meals on moulting, duration of the nymphal stadia, and mortality were studied. The results of these experiments are shown in Tables 16a to 16e.

The size and frequency of blood meals have a profound effect on moulting of the insects in all instars (Figure 13). When the feeding period was 15 seconds on every 4th, 8th, or 16th day the first-instar nymphs did not moult. At a frequency of every 2nd day only about 30% of the insects reached the second instar.

All insects fed for 60 seconds every 2 days reached the third instar but thereafter the number of nymphs that moulted decreased and only 15% of the insects reached the adult stage. When the interval between the blood meals was prolonged to 4 days all the insects reached the second instar and only 25% reached the fifth instar. At a frequency of every 8 days the number of insects that did not moult increased from the first to the fourth instar and 2.5% reached the fourth instar. When the insects were fed for 60 seconds every 16 days 55% reached the second instar.

All insects reached the third instar when fed for 120 seconds over the range of intervals between meals of 2 to 16 days. At a frequency of every 2nd day all the insects reached the adult stage. The number of insects that reached the adult

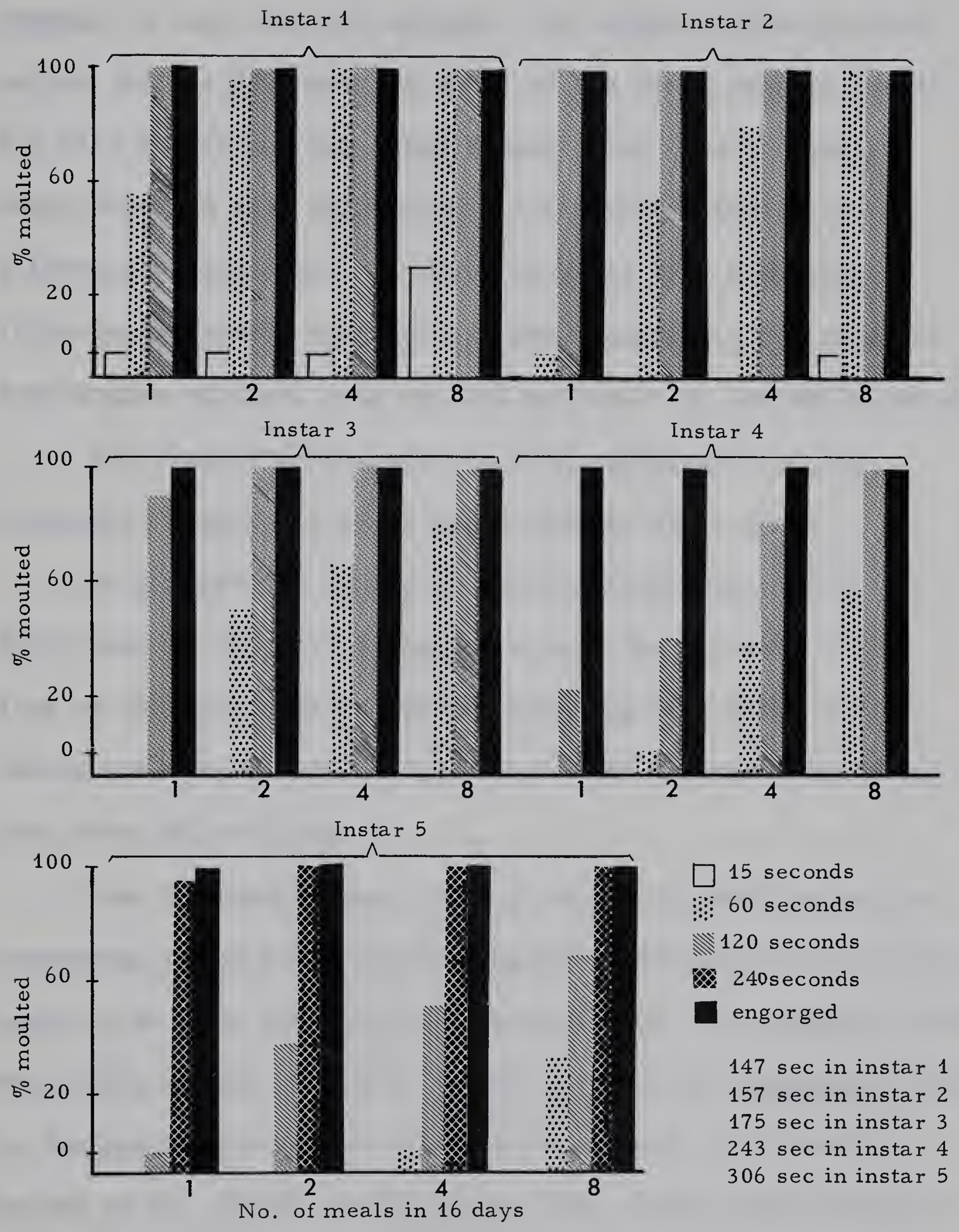

Fig. 13. Effect of the size and frequency of blood meals on moulting in the different instars of C. lectularius.

stage decreased with the increase of the period between meals.

As the insects in the first four nymphal instars can engorge in less than 240 seconds, the effect of this feeding period and its frequency is shown in the fifth nymphal instar. For this experiment the insects were taken from the group which were fed till engorgement to the fourth instar at the different frequencies. As shown in Table 16e, when the fifth-instar nymphs were fed for 240 seconds, in the range of frequencies studied, only one did not moult to the adult stage.

All insects which were fed till engorgement at any frequency reached the adult stage (Tables 16a - 16e).

In general the number of insects which moult after the first meal at any instar increases with the increase in the size of the blood meal. Maximum moulting took place after taking the second meal, if the first meal did not bring about more than 50% moulting.

When the nymphs were given a series of blood meals, the percentage of moulting insects depended upon the size of blood meals as well as the intervals between them. Wigglesworth (1934) was unable to get the fifth instar nymphs of R. prolixus to moult by feeding them a series of small blood meals at intervals. Friend et al. (1965), on the other hand, found that third and fifth instar nymphs of R. prolixus can be induced to moult on a succession of small meals at 30 day intervals. Unfortunately they did not study the effect of the interval between feeding on moulting. My studies also show that successive small meals can

induce moulting and that, in C. lectularius, the interval between feeding is very important. It seems that when the nymphs are fed a series of small meals within certain interval limits they achieve a physiological state after which moulting can be induced by blood meals of small size. Friend et al. (1965) claimed that this might be due to some moulting hormone produced under the influence of small blood meals and stored until it reaches an effective titer and moulting results. They added that the cells may become more responsive to the moulting hormone after they have assimilated the nutrients supplied by small meals. This might be so in C. lectularius because the size and frequency of blood meals has no significant effect on the period between the last meal and the day of moulting.

The frequency of feeding has a profound effect on moulting and this effect is dependent on the size of the blood meal (Figure 13). The smaller the blood meal and the longer the interval between feeding the lower the percentage of the nymphs moulting. This may be due to the effect of the duration of the interval between feeding on the stored moulting hormone suggested by Friend et al. (1965).

The duration of any nymphal stadium increases with the increase in the number of meals required to induce moulting, and with the decrease of frequency of feeding (Tables 16a to 16e). On the other hand, neither the size and interval between blood meals nor the number of meals have a significant effect on the period

between the last meal and moulting. Jones (1930) reported that bedbugs that took two meals during the third stadium moulted at a different time from others, but did not say whether this was earlier or later.

Tables 16a to 16e also show the effect of the size and frequency of blood meals on mortality. Generally, the mortality increased with the decrease in the size and the frequency of blood meals at any instar. Also the longevity of the insects that did not moult at any instar increased with the increase in the size and frequency of feeding.

1.4.2.1 On weight changes during development

Figures 14a to 14f and Table 17 show the relationship between the size and the frequency of blood meals and the difference in body weight of the different instars. The first instar was weighed one day after hatching and the other instars on the day of moulting.

The difference in body weight between any two successive instars increases with the increase in the size of the blood meals and with the decrease in the interval between feeding. Similarly, the ratio of the difference in body weight to the total weight of blood meals usually increases with the increase in the size of the blood meal and its frequency (Table 17). This increase may be due to an increase in the food conversion efficiency with or without a difference in the weight of the unassimilated blood in the gut. The increase in this ratio with the increase in the size of the blood meals when the nymphs were

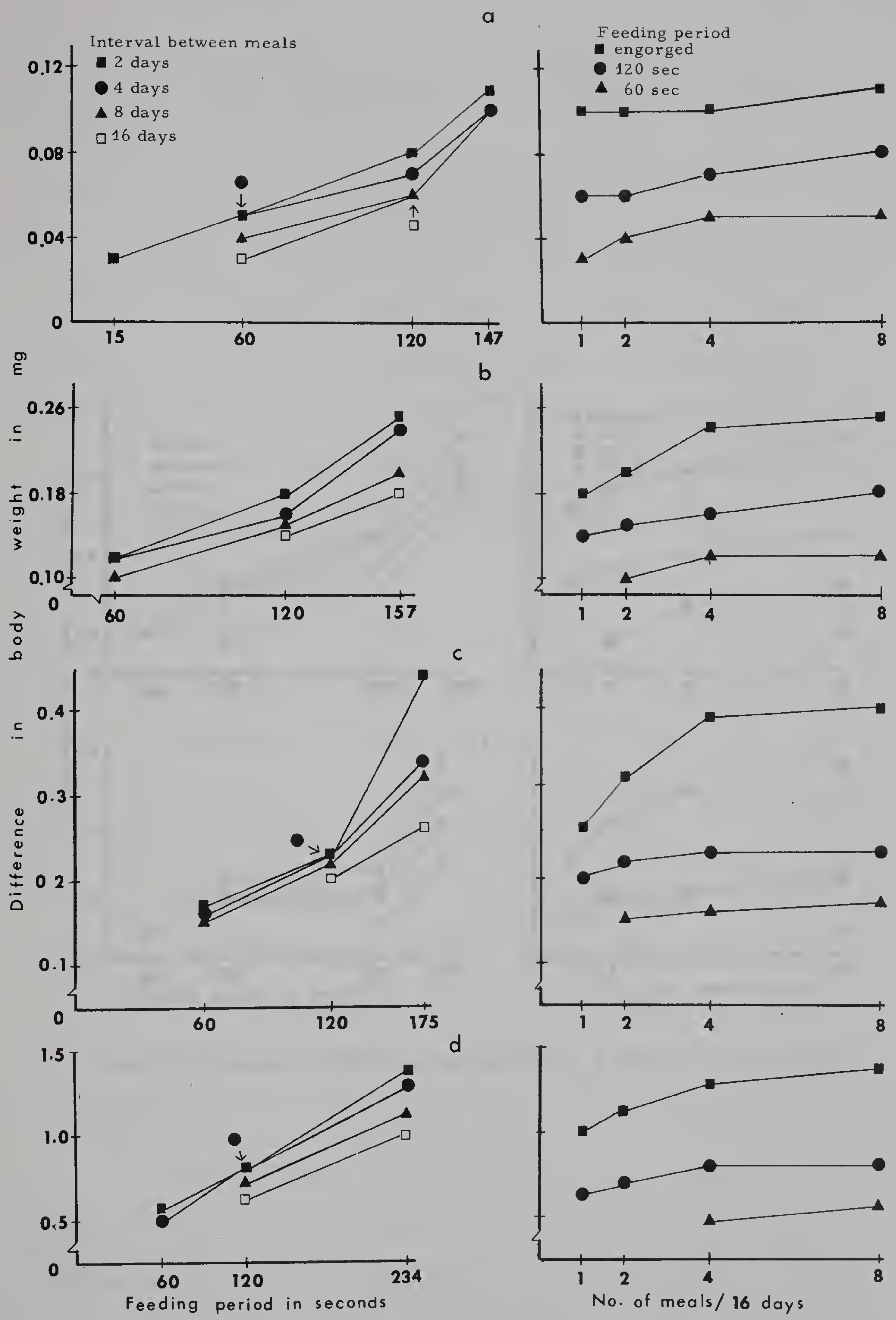

Fig. 14. Effect of the size and frequency of blood meals on the difference in body weight between successive instars of C. lectularius. a. First to second instar; b. Second to third instar; c. Third to fourth instar; d. Fourth to fifth instar.

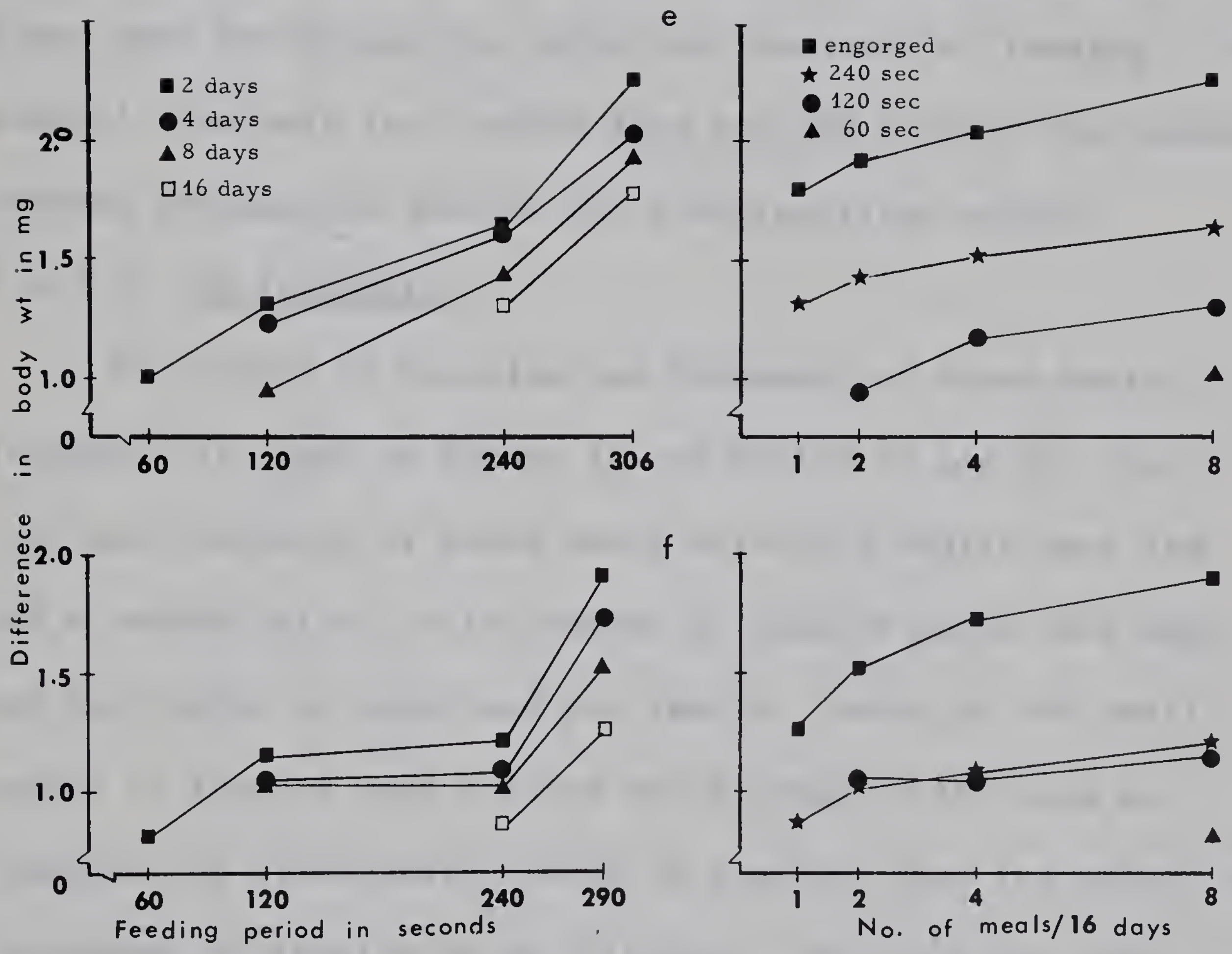

Fig. 14. - "Continued." e. Fifth instar and adult female; f. Fifth instar and adult male.

fed every 16 days is mostly due to an increase in the food conversion efficiency.

1.4.2.2. On the preoviposition period

Figure 15 and Tables 18 and 19 show the effect on the preoviposition period. The effect of frequency of feeding depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. In general, the more the insects feed and the shorter the interval between feeding the shorter the preoviposition period.

1.4.2.3. On fecundity

The effect of the size and frequency of blood meals on fecundity is shown in Figure 16 and Tables 18 and 19. The size and frequency of blood meals which the adults were fed had a marked effect on the number of females which laid eggs and the number of eggs laid per female. Owing to the small number of insects used and the small range in the size and frequency of blood meals, Table 18 does not show the effect on the number of females which laid eggs. On the other hand, it is clearly shown in Table 19 or if the results in Table 18 are combined with those in Table 19. None of the females laid eggs when the feeding period was 15 seconds, at any frequency, nor when fed for 30 seconds every 8th or 16th day. The number of females which laid eggs and the number of eggs laid per female increase with the decrease in the interval between feeding and increases in size of the blood meals. As shown in Table 20 there is a high positive correlation between the weight of blood

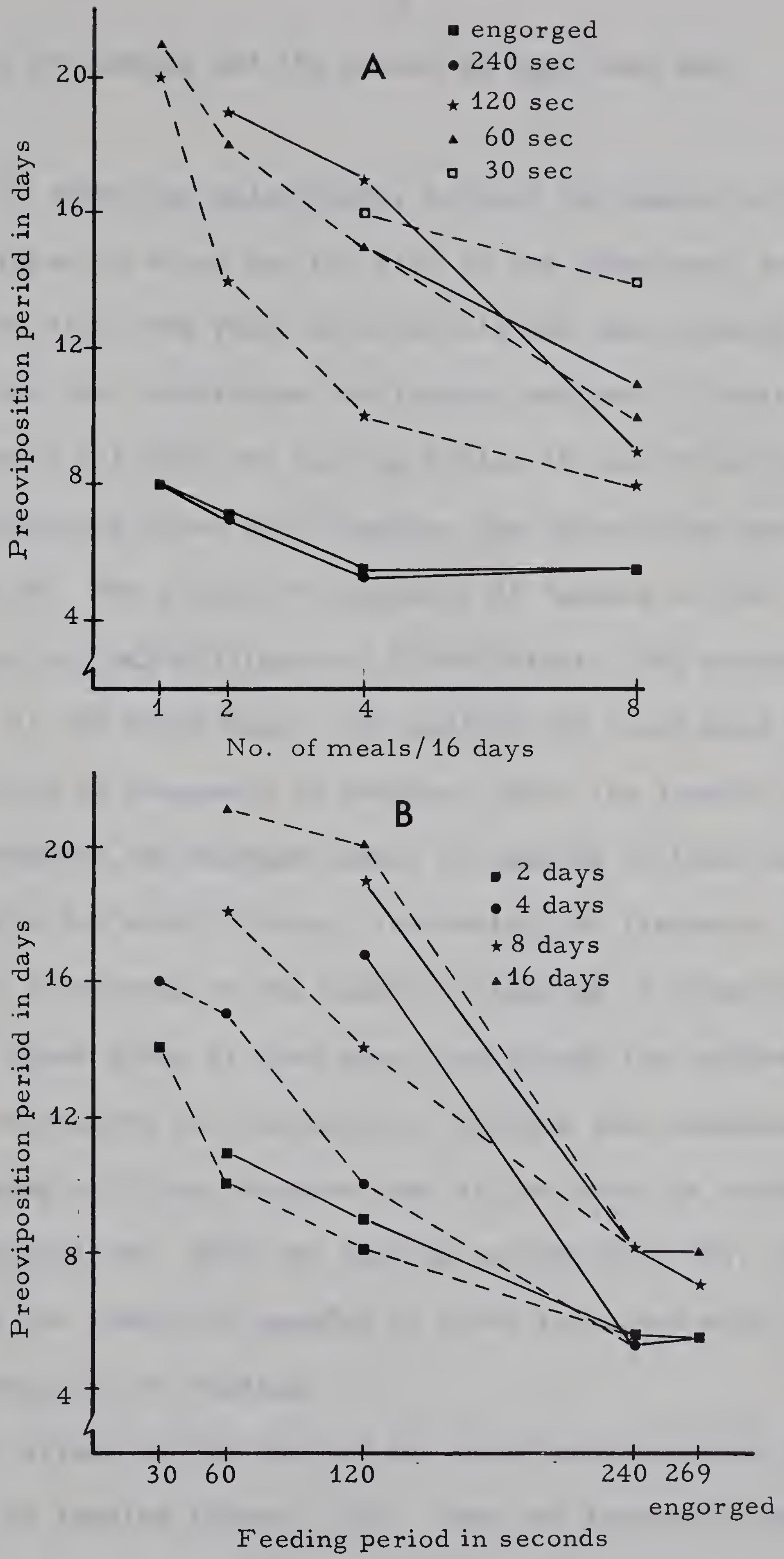

Fig. 15. Effect of the frequency (A) and the size (B) of blood meals on the preoviposition period in C. lectularius.

(--- insects taken from standard culture)

meals taken by the female and the number of eggs laid per female.

Figure 17 shows the relationship between the number of eggs laid per milligram of blood and the size of the blood meal and its frequency. The data from Table 18 alone did not show clearly the relationship and the correlation coefficient was small (Table 20). For this reason I put both the data in Tables 18 and 19 in Figure 17 and when analysing these data together the correlation coefficient was 0.70. The effect of frequency of feeding on the number of eggs laid per milligram of blood (Figure 17A) depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. When the insects were fed till engorgement the maximum number of eggs/mg of blood was when the insects fed every 4 days. Increasing the frequency of feeding causes a decrease in the number of eggs/mg of blood because the amount of blood taken is then more than enough for optimum egg production. Decreasing the frequency of feeding also decreases the number of eggs/mg of blood, because some of the blood is needed for maintenance metabolism. When the feeding periods were 240, 120, 60, and 30 seconds the number of eggs/mg of blood increased with the increase in frequency of feeding.

Also the effect of the size of the blood meals depends upon the frequency of feeding (Figure 17B). When the interval between meals was 2 days the number of eggs/mg of blood increases with the increase in the size of the blood meal and the optimum egg production was when the feeding period is in the range of 120 to 240 seconds. When the interval between feeding was four days

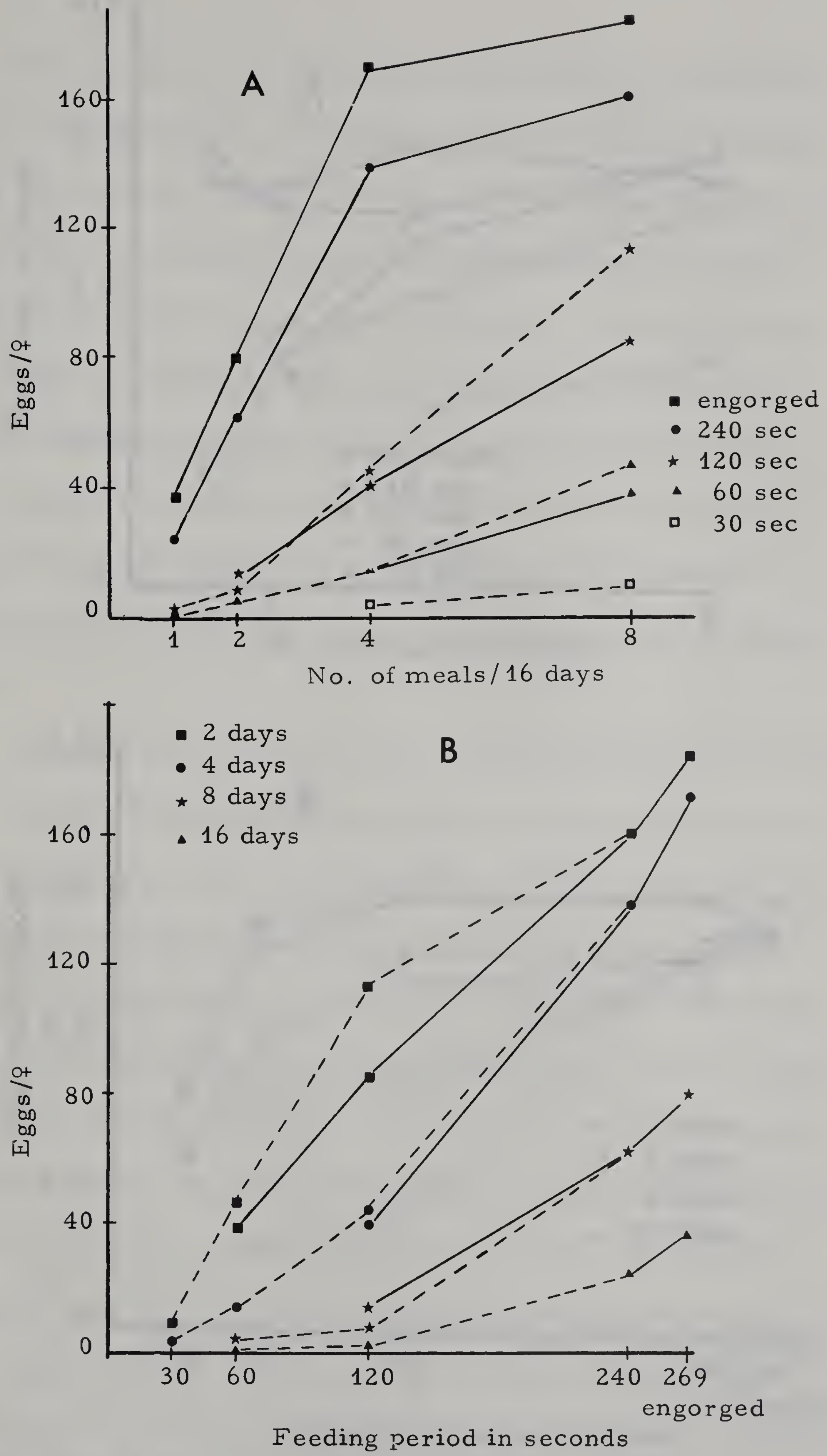

Fig. 16. Effect of the frequency (A) and the size (B) of the blood meals on the number of eggs laid per female C. lectularius.

(--- insects taken from standard culture)

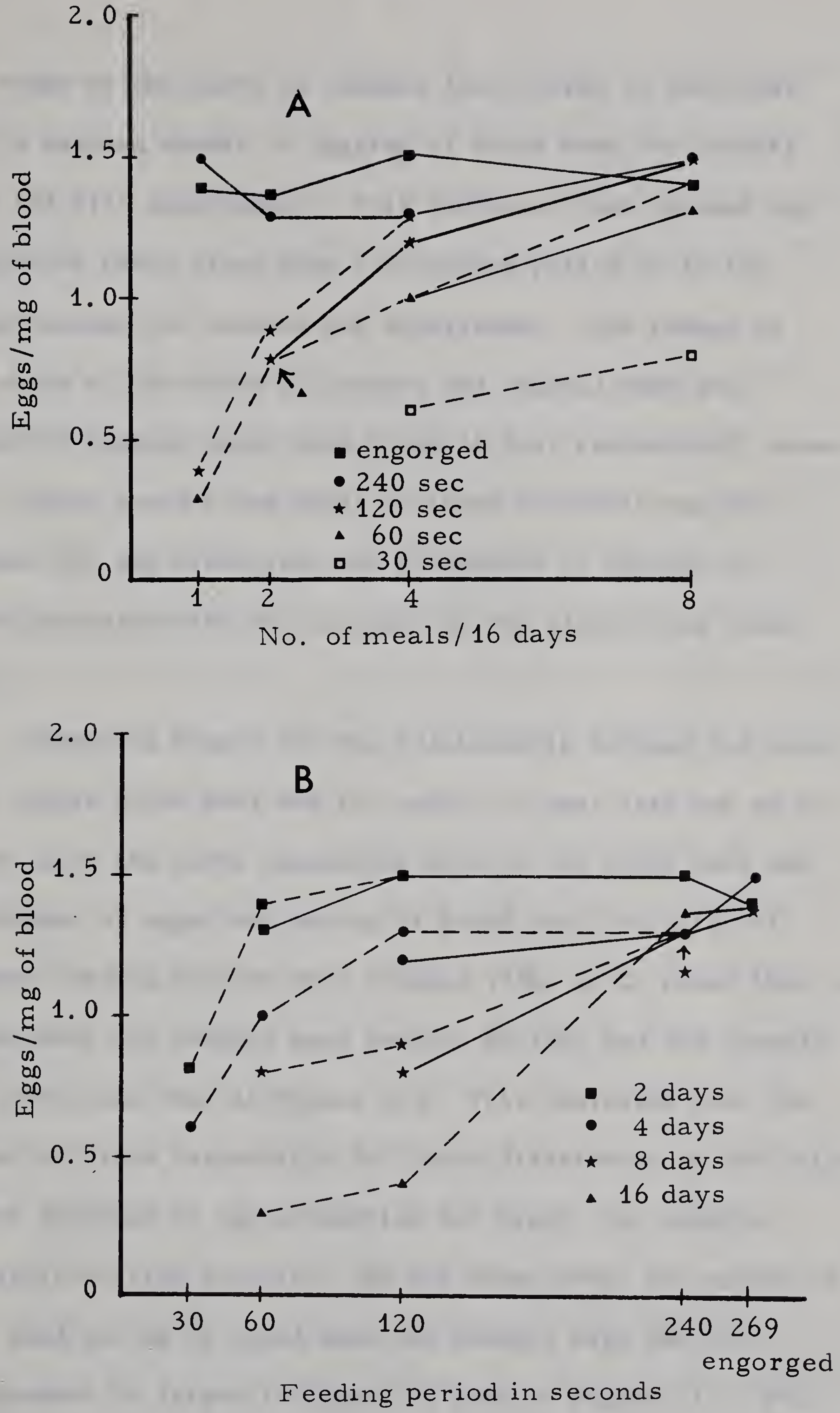

Fig. 17. Effect of the frequency (A) and the size (B) of blood meals on the number of eggs laid per mg of blood in C. lectularius.

(--- insects taken from standard culture)

the shape of the curve is changed from convex to step-like with a maximum number of eggs/mg of blood when the insects were fed till engorgement. This indicates that optimum egg production takes place when the feeding period is in the range between 240 seconds and engorgement. The change in the shape of the curve to concave and sigmoid when the intervals between meals were 8 and 16 days respectively shows that taking these blood meals at these intervals was not optimum for egg production and the number of eggs/mg of blood increases with the increase in the size of the blood meal.

Comparing Figure 10, the relationship between the size of a single blood meal and the number of eggs laid per mg of blood, with the curve connecting size of the blood meal and the number of eggs laid per mg of blood when the interval between feeding was two days (Figure 17 B), it is clear that the values when the insects were fed for 60,120, and 240 seconds are lower than that in Figure 17 B. This indicates that the amount of blood responsible for these differences in the values is not directed to egg production but might, for example, stimulate ovarian activity. On the other hand, the number of eggs laid per mg of blood when the insects were fed till engorgement is larger in Figure 10 than in Figure 17 B. This indicates that the first feeding period required for optimum egg production is in the range between 240 seconds and engorgement.

Table 20 shows that there is no correlation between the weight of the female, after the fifth moult, and the number of eggs laid per female. On the contrary, there is a high positive correlation between longevity and the number of eggs laid per female.

Figure 18 and Tables 21 and 22 show the effect of the mating male's blood meals and their frequencies on the percentage of infertile eggs laid by the females. The smaller the size of the blood meals the larger the effect of frequency of feeding. The correlation coefficient r between the size of the males' blood meals and the percentage of infertile eggs was -0.42 and -0.56 for the data in Table 21 and data in both Tables 21 and 22 respectively.

The results of the experiments on blood intake and egg production in _C_. _lectularius_ are similar to those reported by Roy (1936) and Friend et al. (1965). Roy (1936) worked on _A_. _aegypti_ and found that 0.82 mg of blood was required before the insect would lay eggs. In addition, the number of eggs laid per female was not dependent on the weight of the insect before feeding but there was a good correlation between the size of the blood meal and the number of eggs laid. Friend et al. (1965) found that female _R_. _prolixus_ would not produce eggs until 56.6 mg of blood were fed and the correlation coefficient for the body weight before feeding and egg production was only 0.30.

In some other blood-sucking insects fecundity is influenced

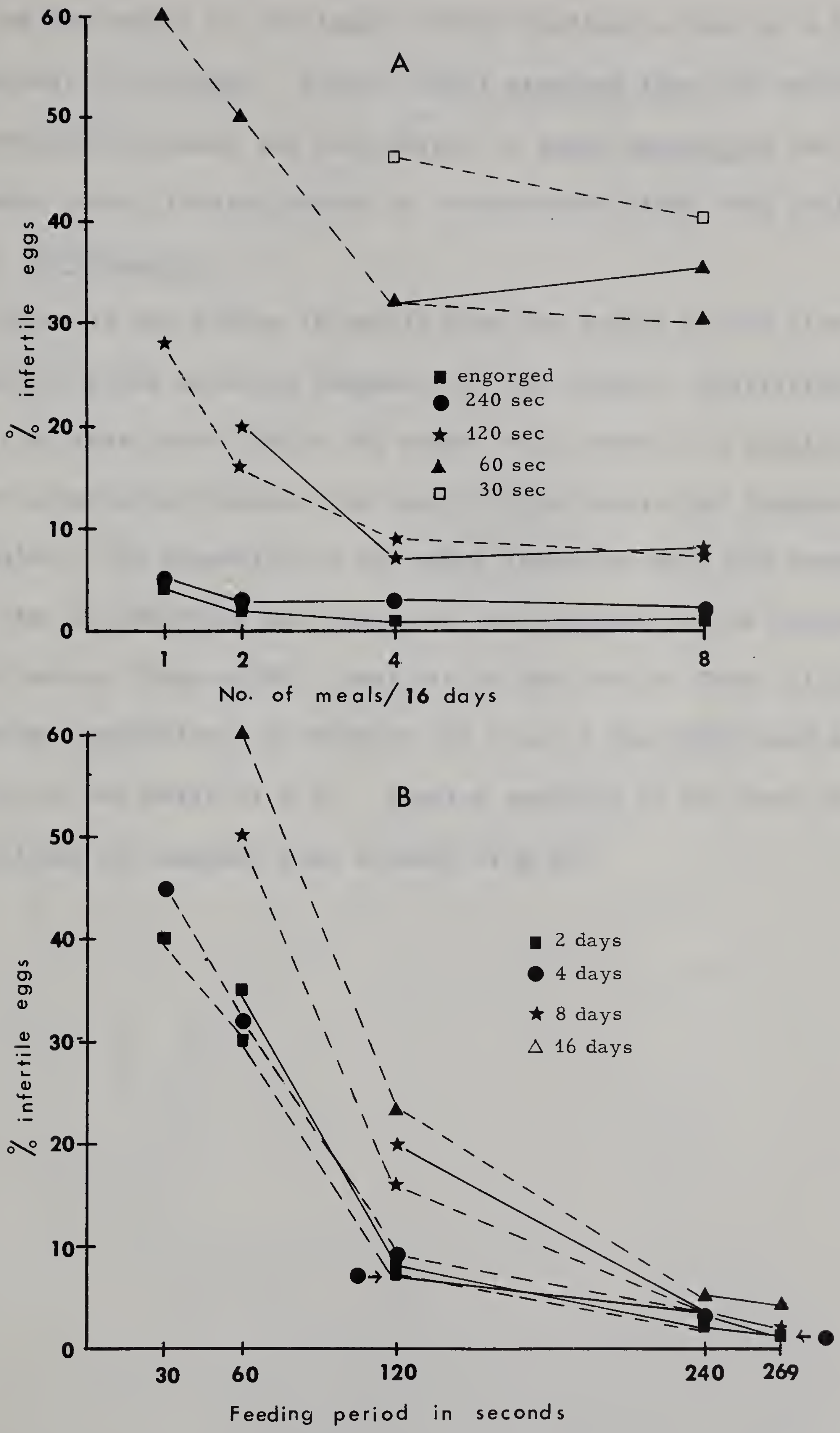

Fig. 18. Effect of the frequency (A) and the size of mating male's blood meals (B) on the percentage of infertile eggs laid by female C. lectularius.

(--- insects taken from standard culture)

mainly by the weight of the female before feeding as long as a certain minimum meal is consumed. Barlow (1955) proposed that the reason for the correlation between the body weight of Aedes hexodontus and fecundity is because larger females tended to consume more blood than smaller ones.

1.4.2.4 On longevity

Figure 19 and Tables 18 and 19 show the effect of the size and frequency of blood meals on longevity of the female. Statistical analysis of these data (Table 20) showed that there is a significant positive correlation between the size of blood meals and longevity of the females. The longevity of the males increases with the increase in the size of the blood meal and with the decrease in the interval between feeding (Figure 20). Analysis of the data in Table 21 showed correlation coefficient (r) between the size of the blood meal and longevity of the males of 0.92. Similar analysis of the data in Tables 21 and 22 together gave a value of 0.84.

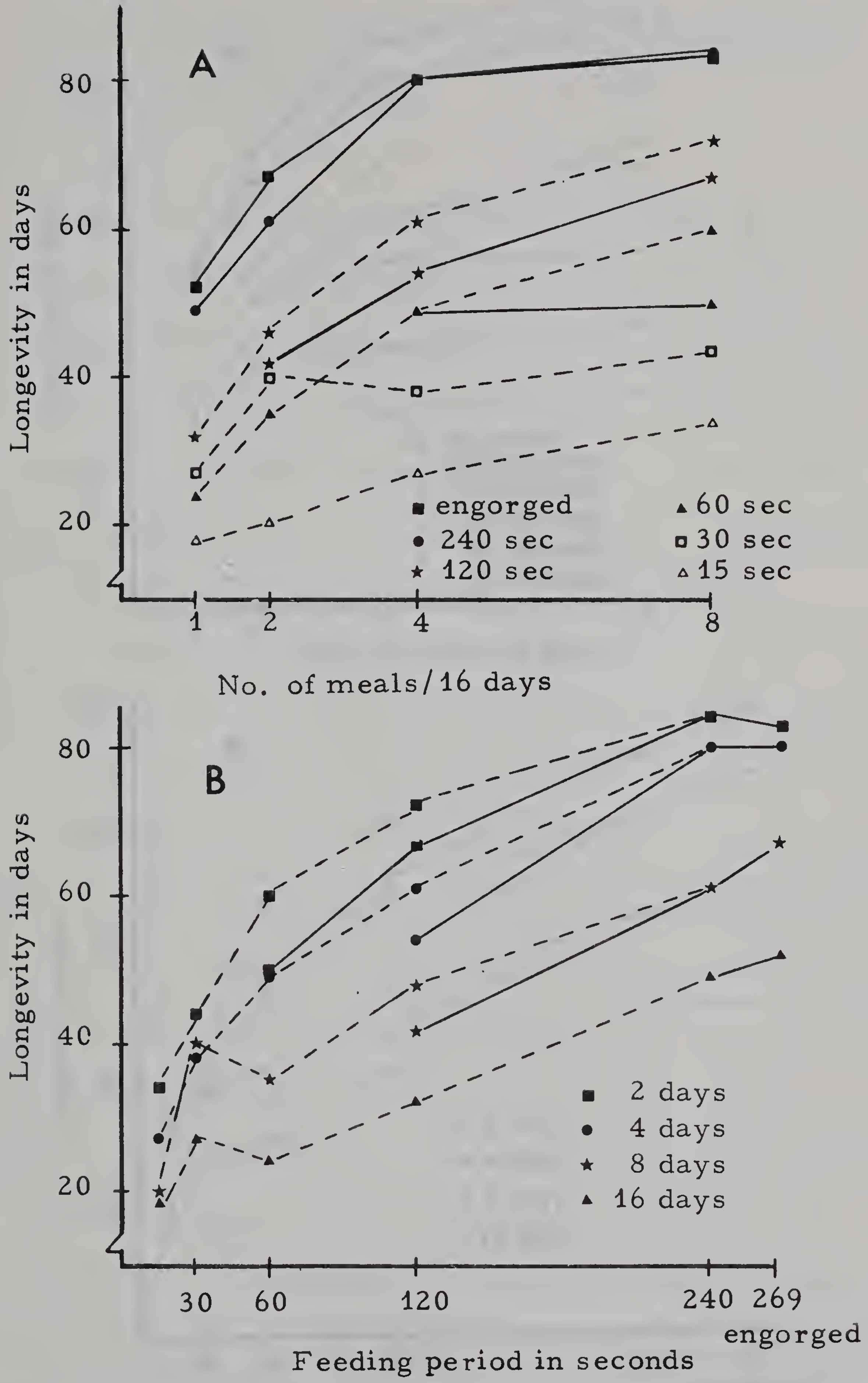

Fig. 19. Effect of the frequency (A) and the size (B) of blood meals on the longevity of female C. lectularius.

(--- insects taken from standard culture)

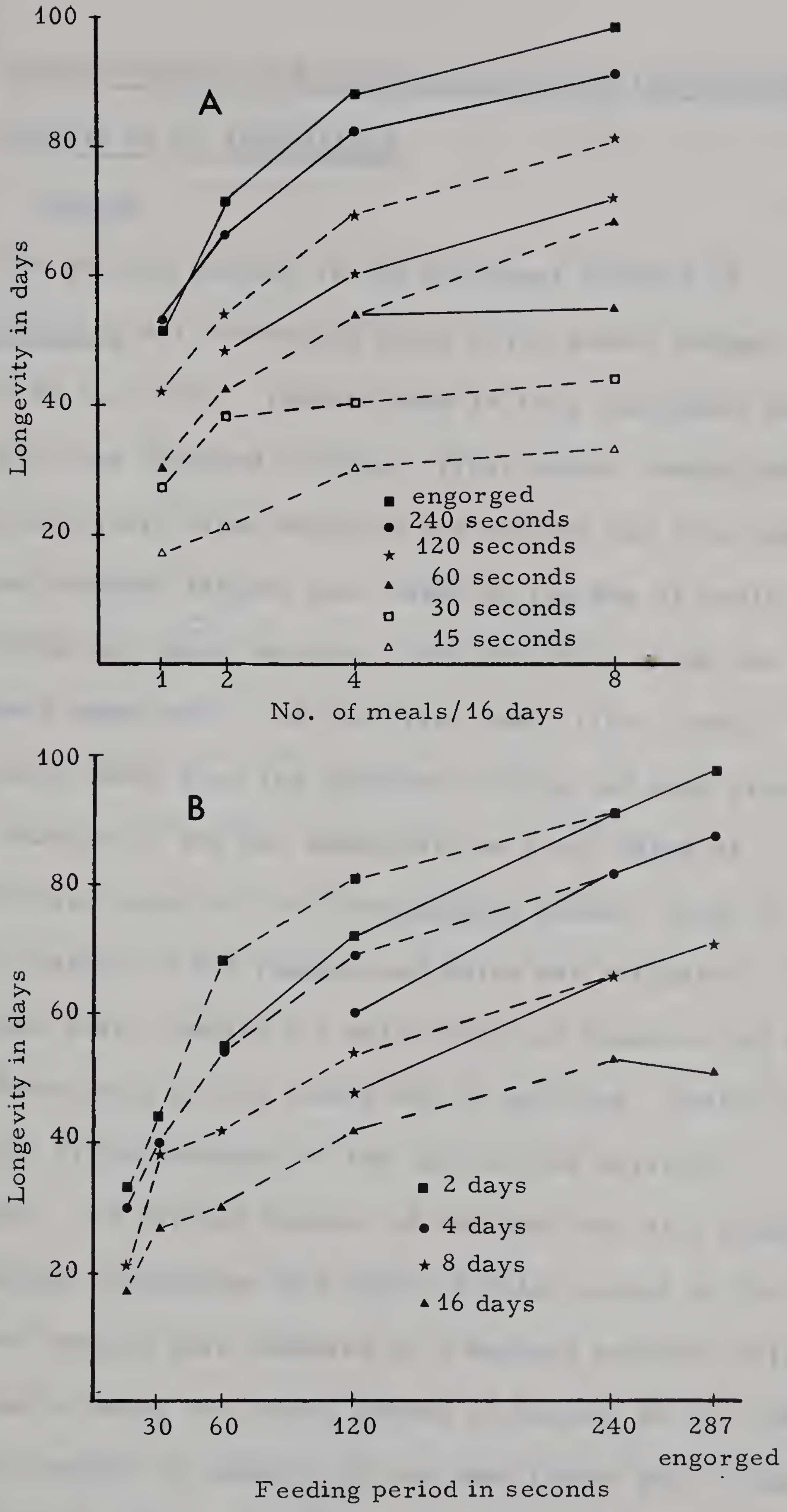

Fig. 20. Effect of the frequency (A) and the size (B) of blood meals on the longevity of the male C. lectularius.

(--- insects taken from standard culture)

1.5. Protein content and respiratory rate in the different instars of *C. lectularius*

1.5.0. Methods

The protein content in the different instars of *C. lectularius* was determined using Folin phenol reagent (Lowrey et al. 1951). Insects used in this experiment were taken from the standard culture. First instar nymphs were taken immediately after hatching and starved for five days. The other nymphal instars were taken on the day of moulting and starved for about ten days. For the adult stage two tests were undertaken. In the first test, fifth instar nymphs were taken from the standard culture and were given a full blood meal and put separately on 2 cm^2 piece of folded filter paper in 2 x 7 cm specimen tubes. After 12 days protein content of the females and males was estimated. In the second test, females and males were put together and given a full blood meal on the second day of moulting. Twelve days later the protein content of the females and males was estimated. The protein content of the eggs was also determined.

Oxygen consumption and carbon dioxide output in the different instars were measured in a Warburg constant volume respirometer using the direct method of Umbreit et al. (1964). A certain number of insects, of the same instar and of known weight, were put in the flask together with a small piece of folded paper. Carbon dioxide was removed by filter paper soaked with 0.2 ml of 10% potassium hydroxide in the center

well of the flask. Insects used were taken from the standard culture one day after moulting and were given a blood meal before starting the experiments. Analysis of the data was undertaken for determining the volume of oxygen consumed per insect per hour, volume of oxygen consumed per milligram of body weight per hour, and the volume of carbon dioxide output per insect per hour. The respiratory quotient in the different instars was determined.

1.5.1. Protein content

The protein content in the different instars of *C. lectularius* is shown in Figure 21 and Table 23. The average weight of protein per insect increases from the first nymphal instar to the adult stage. The weight of protein per female was greater than that per male. Females which were kept separately had a higher protein content than those which were kept with males. Oviposition seems to be responsible for this difference because the females which were kept with males laid eggs while those which were kept alone did not. Similarly, the difference in protein content of the males may be due to sperm transfer as well as the mechanical aspects involved in courtship and mating. The percentage of protein in the body was in the range of 21.8 to 27% in the nymphal instars. In the females it was 26.1 in those which were kept alone and 18.2% in those which were kept with males. Similarly the percentage of protein in the males was 27.2 in those kept alone and 25.4% in those kept with females.

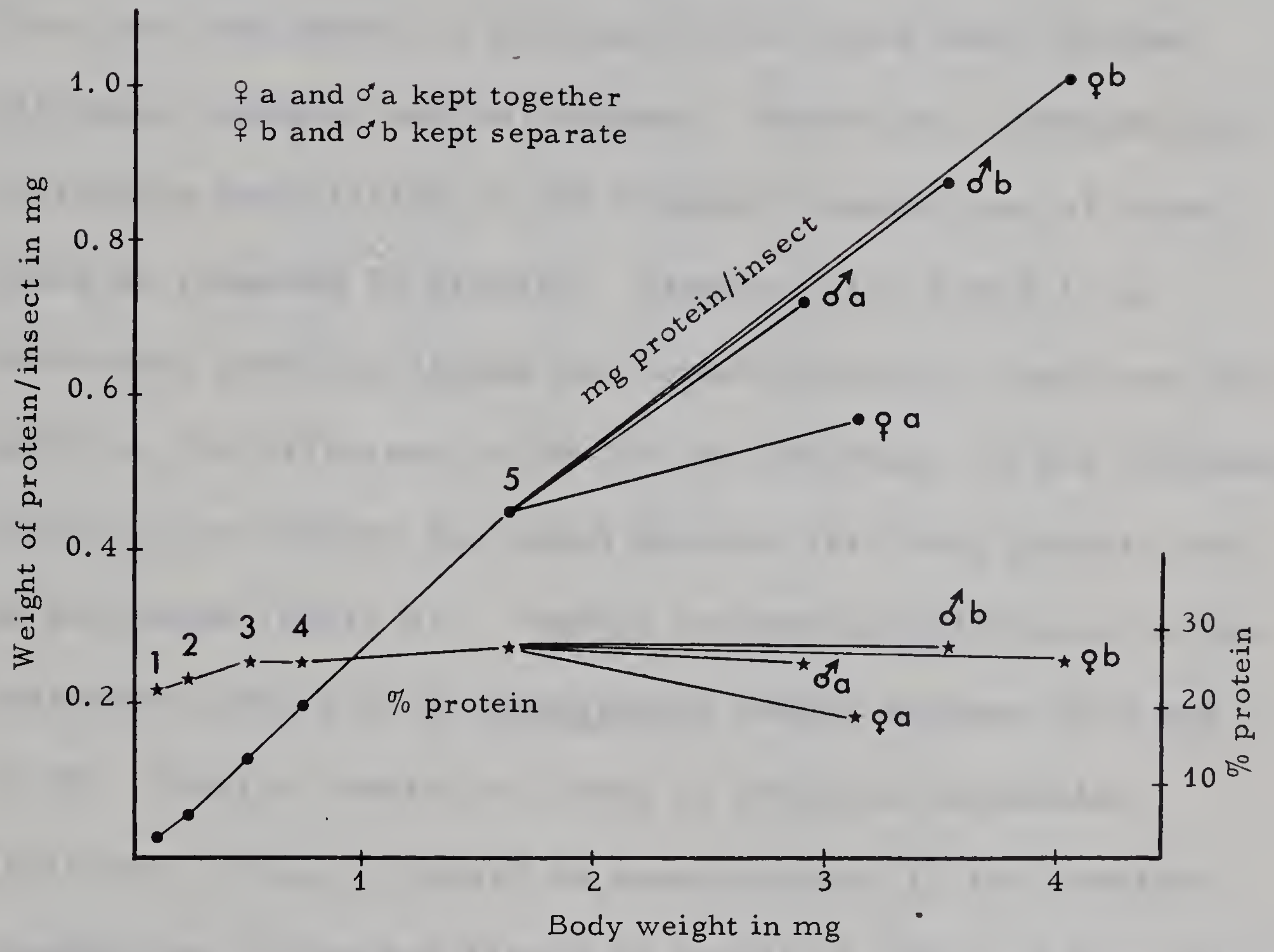

Fig. 21. Relationship between the body weight and protein content in the different instars of *C. lectularius*.

Spector (1956) gave the following chemical composition of the human blood:

water	83.000 g/ 100 ml
total protein	21.800 g/ 100 ml
lipids	0.560 g/ 100 ml
carbohydrates	0.439 g/ 100 ml.

From this the amount of protein in the blood meal, in the different instars, was calculated. Lipids and carbohydrates contribute very little in the chemical composition of human blood as compared to protein. Assuming that there is no conversion from the lipids and carbohydrates of the blood into protein, the efficiency by which the bed-bugs, in the different instars, can convert the blood protein into body protein can be estimated (Table 8). Protein conversion efficiency in the different instars of *C. lectularius* ranged between 28.0 and 65.3%. Similar results to those of the food conversion efficiency (Table 7) would be expected only if the chemical composition of bed-bug tissue is similar to that of human blood.

1.5.2. Rate of respiration

The respiratory rates in the different instars of *C. lectularius* are shown in Figure 22 and Table 24. The volume of oxygen uptake per insect per hour increases from the first nymphal instar to the adult stage. Oxygen uptake by the females was greater than by the males. The oxygen uptake, expressed as microliters/milligram of body weight/hour, was the same in the first two nymphal instars and then increased to a maximum in the

Table 8 - Protein conversion efficiency in the different instars of *C. lectularius*.

Instar	Wt of blood meal in mg	Protein content of blood meal in mg	Increase in body protein content in mg	Protein conversion efficiency %
First	0.26	0.054	0.035	65.3
Second	0.62	0.128	0.070	54.8
Third	1.09	0.225	0.068	30.3
Fourth	2.86	0.589	0.256	43.4
Fifth ♀	5.64	1.162	0.603	51.9
Fifth ♂	5.42	1.117	0.313	28.0

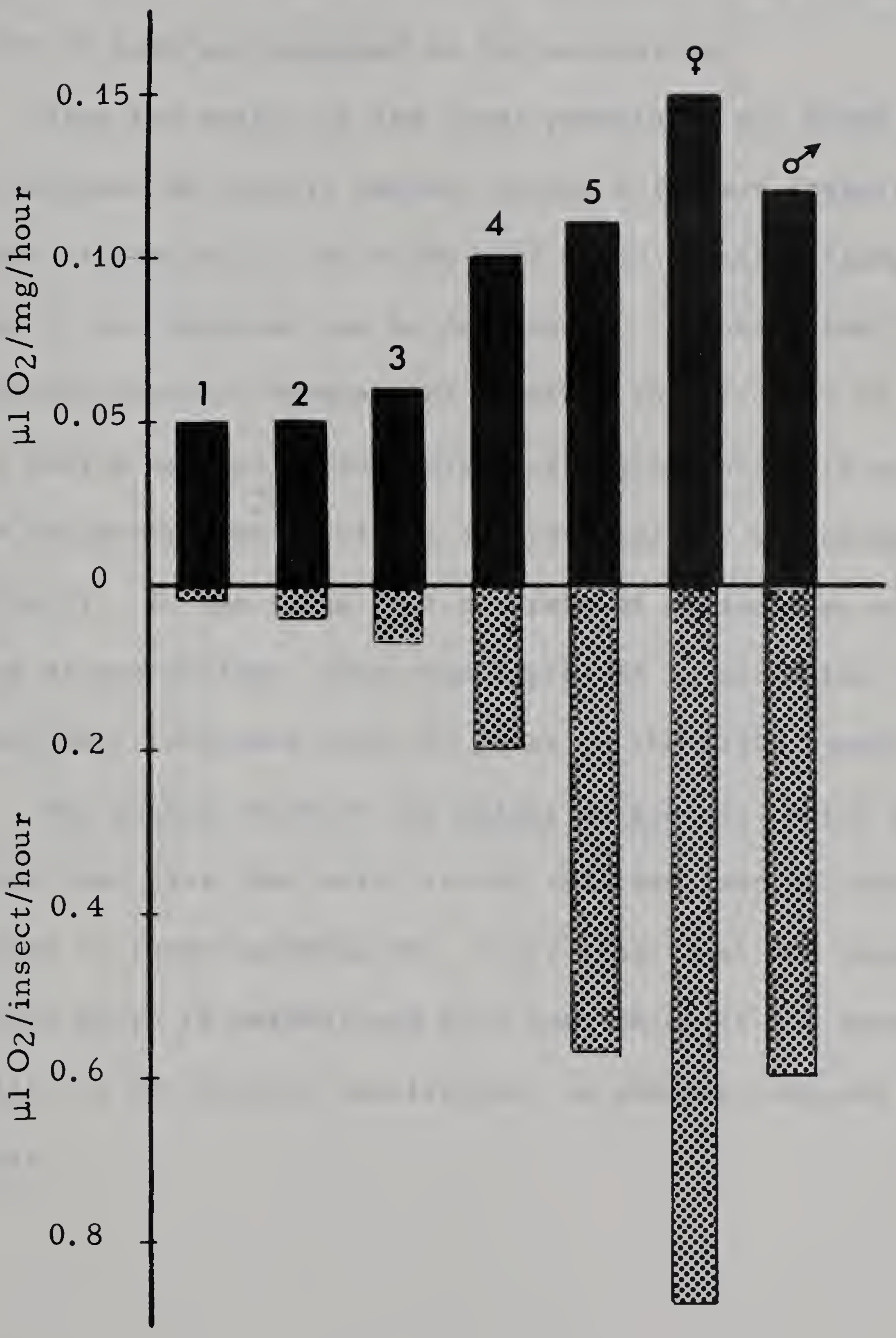

Fig. 22. Oxygen uptake in the different instars of *C. lectularius*.

adult stage. The respiratory quotient in the different instars ranges between 0.88 and 0.95 which indicates that either protein or fat or both are included in the metabolism.

From the weight of the total protein in the blood meal and the increase of protein content in the different instars, the weight of protein in the blood meal which is metabolized plus that which is not absorbed can be determined. Assuming that there is no interconversion between the chemical constituents of the blood meal during metabolism the weight of protein which is metabolized plus the protein which is not absorbed per day was estimated (Table 9). It was found that the rate of metabolism, expressed as mg of protein/day, like that expressed as microliter oxygen/insect/day, increases from the first to the fifth nymphal instar.

The method by which the weight of protein, which is metabolized plus that which is not absorbed, was estimated in the females is shown in Table 10. It is clear that the weight of protein which is metabolized plus that which is not absorbed, similar to the rate of respiration, is greater than in any nymphal instar.

Table 9 - Rate of metabolism in the different nymphal instars of *C. lectularius*.

Instar	Wt of protein metabolized plus protein not absorbed in mg	Mean longevity in days	Wt of protein metabolized plus protein not absorbed per day in mg	Rate of respiration µl O_2/insect / day
First	0.019	34.9	5.4×10^{-4}	0.48-0.96
Second	0.058	63.6	9.1×10^{-4}	0.96-1.68
Third	0.157	63.9	2.5×10^{-3}	1.68-4.80
Fourth	0.333	68.9	4.9×10^{-3}	4.80-13.68
Fifth ♀	0.559	89.1	6.3×10^{-3}	13.68-21.12
Fifth ♂	0.804	89.1	9.0×10^{-3}	13.68-14.40

Table 10 - A partial protein budget in the adult female *C. lectularius*.

Weight of blood meal	6.48 mg
Protein content of the blood meal	1.40 mg
Weight of protein/egg	0.034 mg
Number of eggs laid/female	11.55 eggs
Weight of protein in all the eggs	0.393 mg
Weight of protein metabolized + protein not absorbed	1.007 mg
Mean longevity	39.6 days
Weight of protein metabolized + not absorbed	$2.5\text{x}10^{-2}$mg/day
Respiratory rate	0.88 µl O_2/female/hour

1.6. General discussion and summary

The size of the blood meal has its effect in two ways. The first one is the volume of the blood ingested that causes either stretching the abdomen and stimulates moulting in the nymphal instars or stimulates oviposition in the adult females. The second is the quantity of nutrients in the blood ingested and its effects on development or reproduction. It seems that taking a threshold quantity of blood as a single meal is more important to C. lectularius than taking the same quantity over a period of time in more than one blood meal. Also the blood of different hosts may have its effect on C. lectularius through the differences in the nutritive value of blood.

The results of the effects of the size and fequency of blood meals on C. lectularius can be summarized as follows:

(1) Moulting can be induced in all the nymphal instars by blood meals smaller than the full blood meal and there is always positive correlation between the size of the blood meal and the percentage of insects moulting.

(2) The duration of the nymphal stadia decreases with the increase in the size of the blood meals. It also decreases with the decreases in the number of successive meals required to induce moulting. Neither the size and interval between blood meals nor the number of meals have a significant effect on the period between the last meal and the day of moulting.

(3) The food conversion efficiency in the different instars varies between 25.6 and 37.0% and the third instar is less efficient than the others. It takes about 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%.

(4) The increase in the size of the females' blood meals causes a decrease in the preoviposition period. The size of the male's blood meal has no significant effect on the preoviposition period of a mated famale with him.

(5) No eggs were produced by unfed females. The more the females were fed the larger the number of eggs laid per female. There is no significant correlation between the body weight of the female and the number of eggs laid. There was no effect of the size of the mating male's blood meal on the percentage of females laying eggs. The percentage of sterile eggs increased with the decrease in the size of the mating males' blood meals.

(6) There is always a significant correlation between the size of the blood meals and the longevity of any instar.

(7) From these studies it seems important to study the role of crowding on frequency of mating and the traumatic effects of repeated mating.

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PART 2. EFFECTS OF POPULATION DENSITY ON CIMEX LECTULARIUS L.

2.0. Introduction

Many studies have been made of the environmental factors that influence development and behaviour of *C. lectularius*. Also, quantitative studies of natural populations have been conducted by Mellanby (1939b), Omori (1941), and Johnson (1942). Although the effect of population density on the physiology and ecology of many insects has received much attention by many investigators, no detailed investigations have been carried out on *C. lectularius*. High population density, or overcrowding, is often accompanied by a shortage of food. In part 1 of this thesis the effect of food quantity free from the effect of population density was studied. The aim of the present studies is to try to separate the effect of density itself from that of food quantity.

Many observations have been reported on the effect of population density on insects. From these observations it can be recognized that overcrowding has a detrimental effect on insects and it manifests itself in various ways; slower growth rate, increased mortality, smaller adults, and lower fecundity (Pearl and Parker 1922, Chapman 1928, Putnam 1934, Bodenheimer 1938 and 1955, MacLagen and Dunn 1936, Crombie 1942, Robertson and Sang 1944, Terzian and Stahler 1949, Bar-Zeev 1957, Wada 1965). Most of the investigators attributed the effect mainly to the mechanical disturbance of the insects by each other. On

the other hand, some investigators demonstrated that most of these effects are dependent on the quantity and quality of food.

2.1. Methods

Density ranging between 2 and 128 insects per surface area of 8 cm^2 of a piece of folded filter paper (2 x 2 cm) in 2 x 7 cm specimen tube was studied. Relating the density to the surface area of the folded filter paper is more important than relating it to the volume of the specimen tube because bed bugs are dorso-ventrally flattened, always aggregate on the surface of the folded filter paper, and thus essentially live in a two dimensional habitat. Data representing a density equal to 1 was taken from part 1 when the insects were fed till engorgement every 2 days. Eggs were taken from the standard culture and put in 4 x 4 x 1.5 cm plastic boxes. First instar nymphs were taken as soon as they hatched and were put in 7 x 2 cm specimen tubes together with 2 x 2 cm folded filter paper. Eight experiments were conducted in which the number of nymphs per tube was 2,4,8,16,32,64 and 128 respectively. In all the experiments the insects were allowed to feed on the second day after hatching and twice every week thereafter. For feeding, the insects from each tube were transferred to a 4 x 4 x 1.5 cm plastic box and were allowed to feed on human blood through the organdie covering a 3 cm diameter hole in the lid of the plastic box. Observations were carried out daily and the effects of the population density on the duration of the nymphal stadia, preoviposition period, fecundity, longevity, and mortality rate were studied.

With a constant food supply for the insects in all the experiments, the effect of population density itself could be due to either visual stimulus, olfactory stimulus, contact stimulus or combinations of these stimuli. All the experiments were

conducted in the dark and the insects were exposed to the light only ten minutes for examination to rule out the visual stimulus. An additional experiment was conducted to test whether the effect of population density is due to olfactory stimulus or a contact one. In this experiment 40 plastic boxes, similar to those used for feeding, were used. The lids of each 2 were glued together forming a combination of 2 chambers separated by the organdie on the inside of the lids. In one of these chambers a single first instar nymph was put with a piece of folded filter paper. In the other chamber 64 first instar nymphs were put. The insects were fed twice every week on human blood and the effect on the duration of the nymphal stadia of the single nymph was recorded. After the fifth moult two insects of opposite sex were put in one chamber instead of one insect and the effects on preoviposition period, fecundity and longevity were studied. All the experiments were done at 80 F and 75% R.H.

2.2. On the duration of the nymphal stadia

The effect of population density on the duration of the nymphal stadia is shown in Figure 23 and Table 25. Population density does not show a clear effect on the duration of the first nymphal stadium as it ranges between 3.6 and 4 days. On the other hand, the duration of the other four stadia decreased with the increase of population density. Olfactory stimulus does not seem to be involved in the effect on the duration of the nymphal stadia because there is no significant difference between the results of the experiment with the double chamber combination and those at

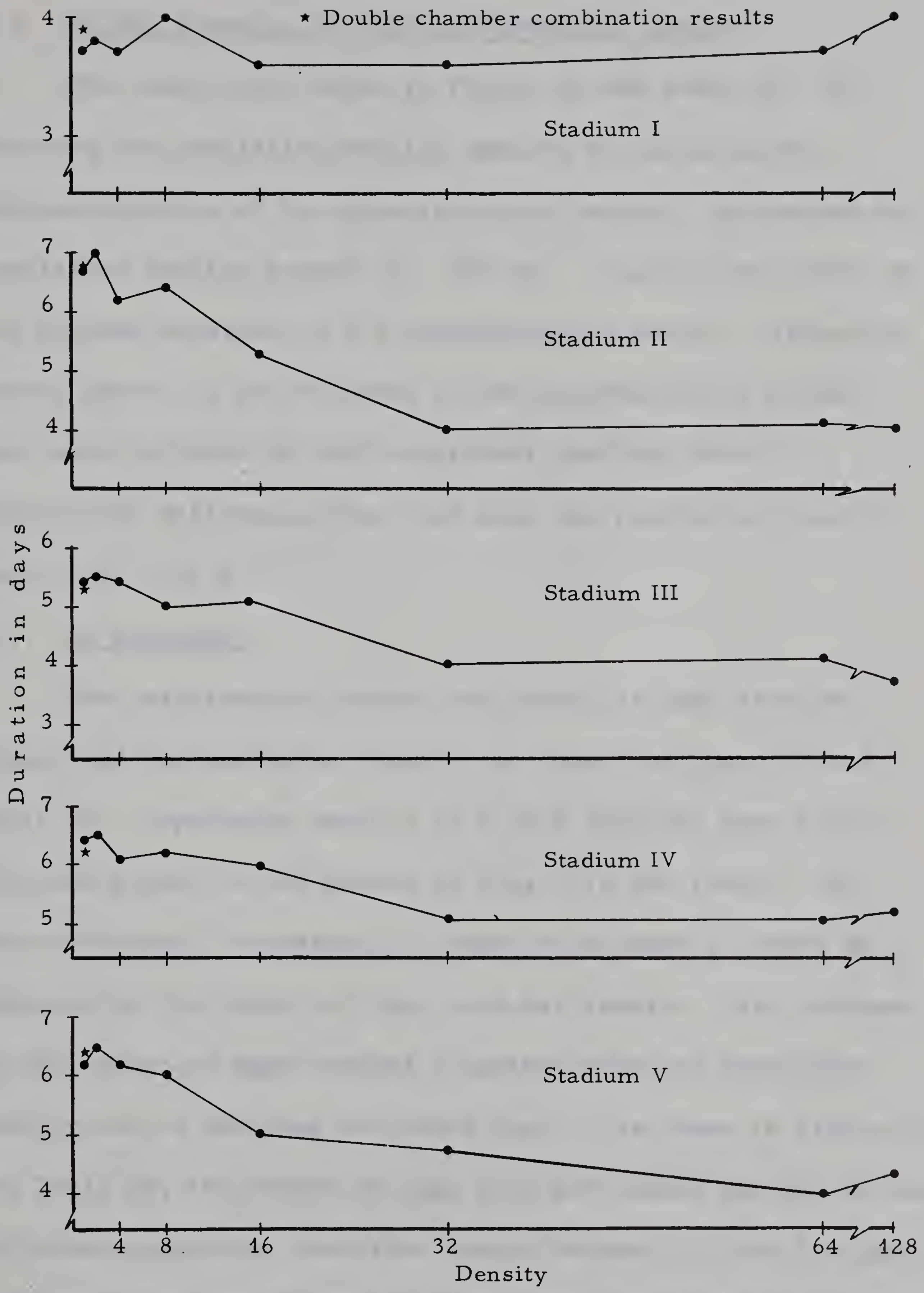
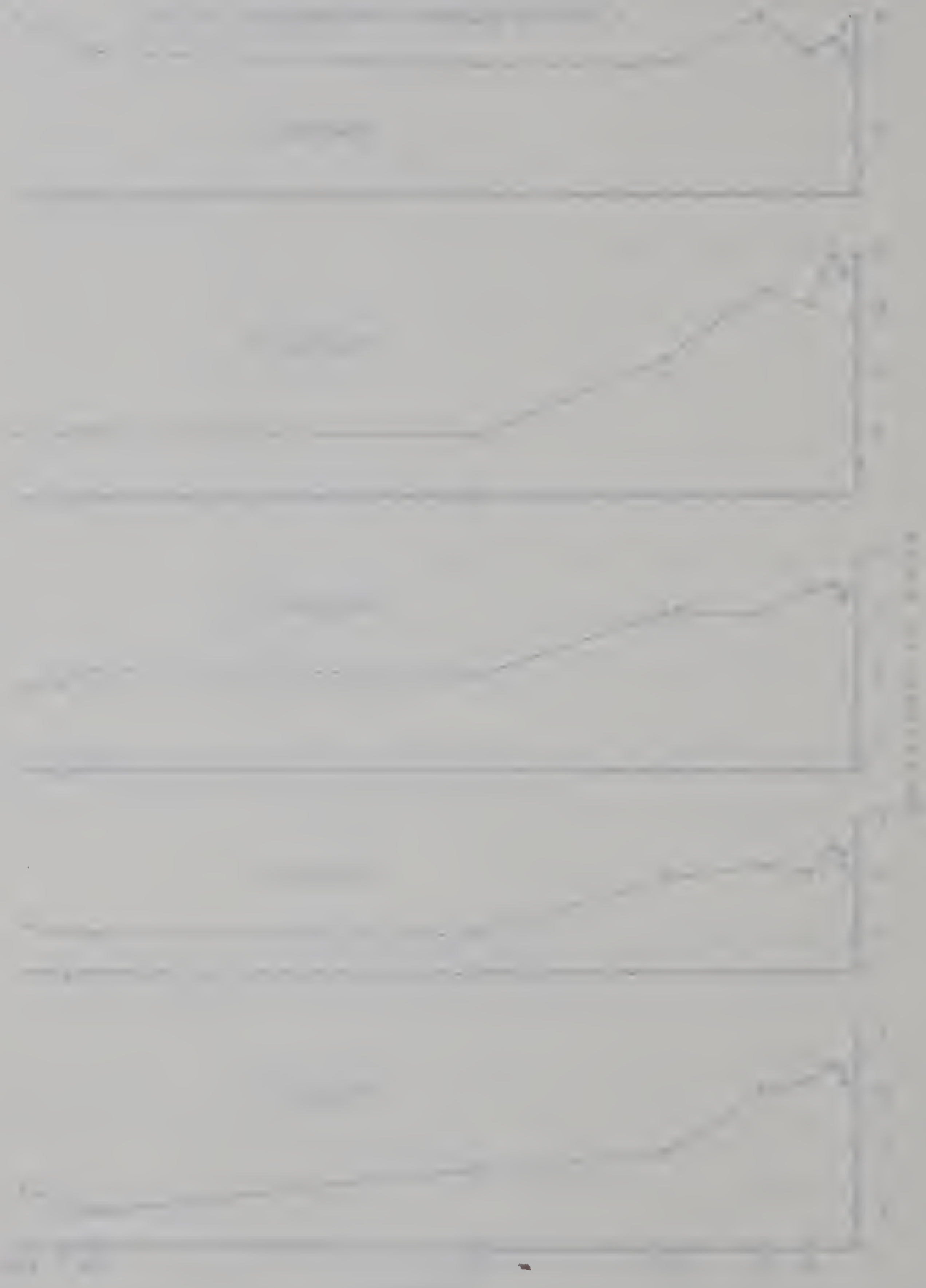


Fig. 23. Effect of population density of C. lectularius on the duration of the nymphal stadia.



density equals to 1 and 2 (Figure 23).

2.3. On the duration of the preoviposition period

The results are shown in Figure 24 and Table 26. Increasing the population density appears to decrease the minimum duration of the preoviposition period. Increasing the population density beyond 32 has no significant effect on the minimum duration of the preoviposition period. Olfaction has no effect on the duration of the preoviposition period; the value obtained in this experiment does not show a significant difference from that when the population density was either 2 or 4.

2.4. On fecundity

The relationship between the number of eggs laid per female and the population density is shown in Figure 25 and Table 26. Population density of 2 or 4 does not have a significant effect on the number of eggs laid per female. On the other hand, increasing the population density causes an increase in the number of eggs laid per female. This increase in the number of eggs reached a maximum when the population density was 64 and then decreased again. As shown in Figure 25 and Table 26, the number of eggs laid per female per day in the different population densities ranged between 2.1 and 2.4 eggs per female per day. Figure 25 also shows that there is no olfactory stimulus involved in the effect of the population density on fecundity of the females.

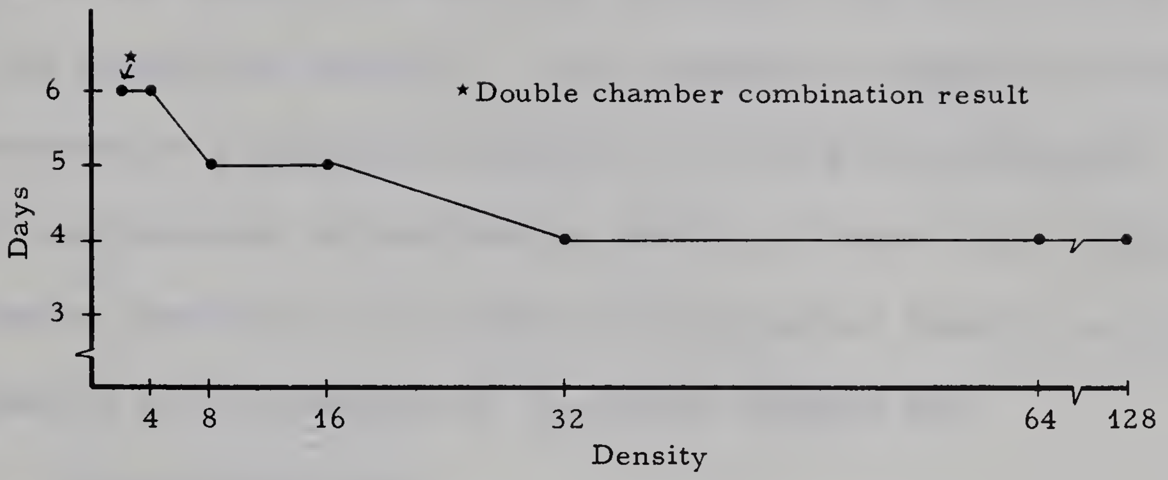


Fig. 24. Effect of the population density of C. lectularius on the duration of the preoviposition period.

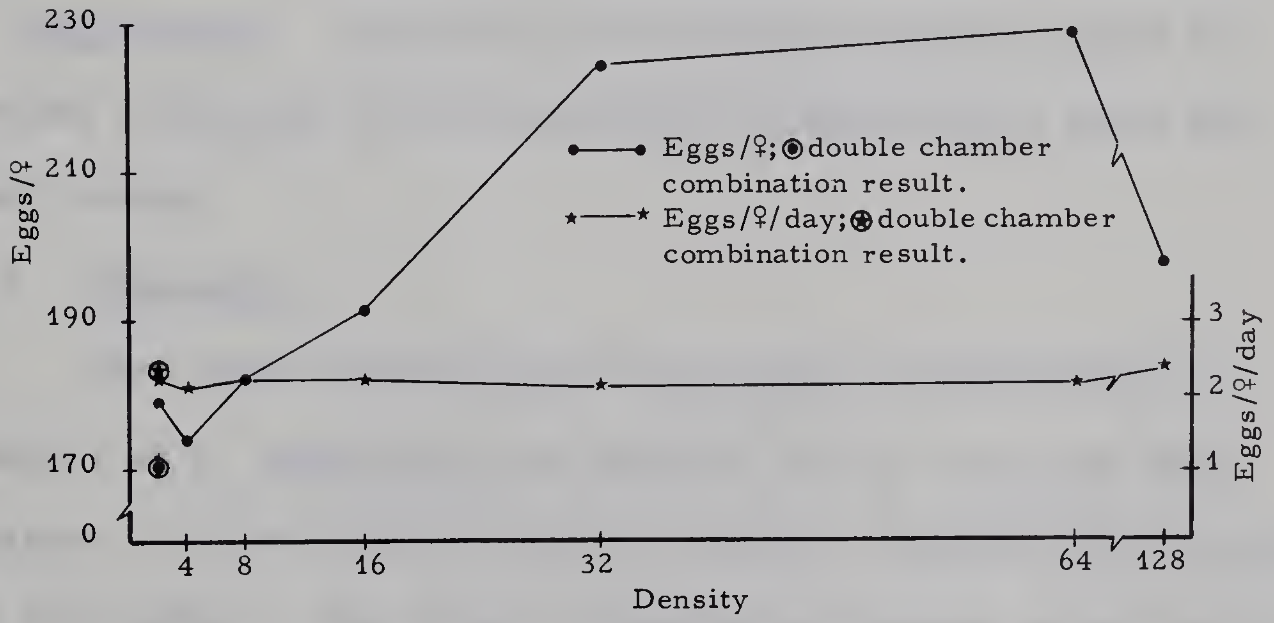


Fig. 25. Effect of population density of C. lectularius on the number of eggs laid per female and the number of eggs laid per female per day.

2.5. On longevity of the adult stage

Figure 26 and Table 26 show the effect of population density on the longevity of the adult stage. The longevity of both the female and the male increases with the increase in the population density. This increase in longevity reaches a maximum at a population density of 32 and then decreases with the increase in population density. There is no olfactory stimulus involved in the effect of population density on the longevity of the females or the males (Figure 26).

2.6. On mortality rate

Table 11 shows the effect of the population density on the percentage of mortality in the different instars of *C. lectularius*. Increasing the population density beyond 8 causes a decrease in the percentage of insects that reach the adult stage.

2.7. Discussion

From these investigations the effect of population density on *C. lectularius* was apparent and it was clear that neither a vision stimulus nor an olfactory stimulus was involved in that effect. The effect of population density on the duration of the nymphal stadia, preoviposition period, fecundity, and longevity reaches an optimum at a population density of 32 to 64 insects per 2 cm^2 folded filter paper in 2 x 7 cm specimen tube. Population density seems to influence the bed-bug through the stimulations of increased mutual contact. This influence was also suggested for mosquito larvae by Ber-Zeev (1957) and Shannon

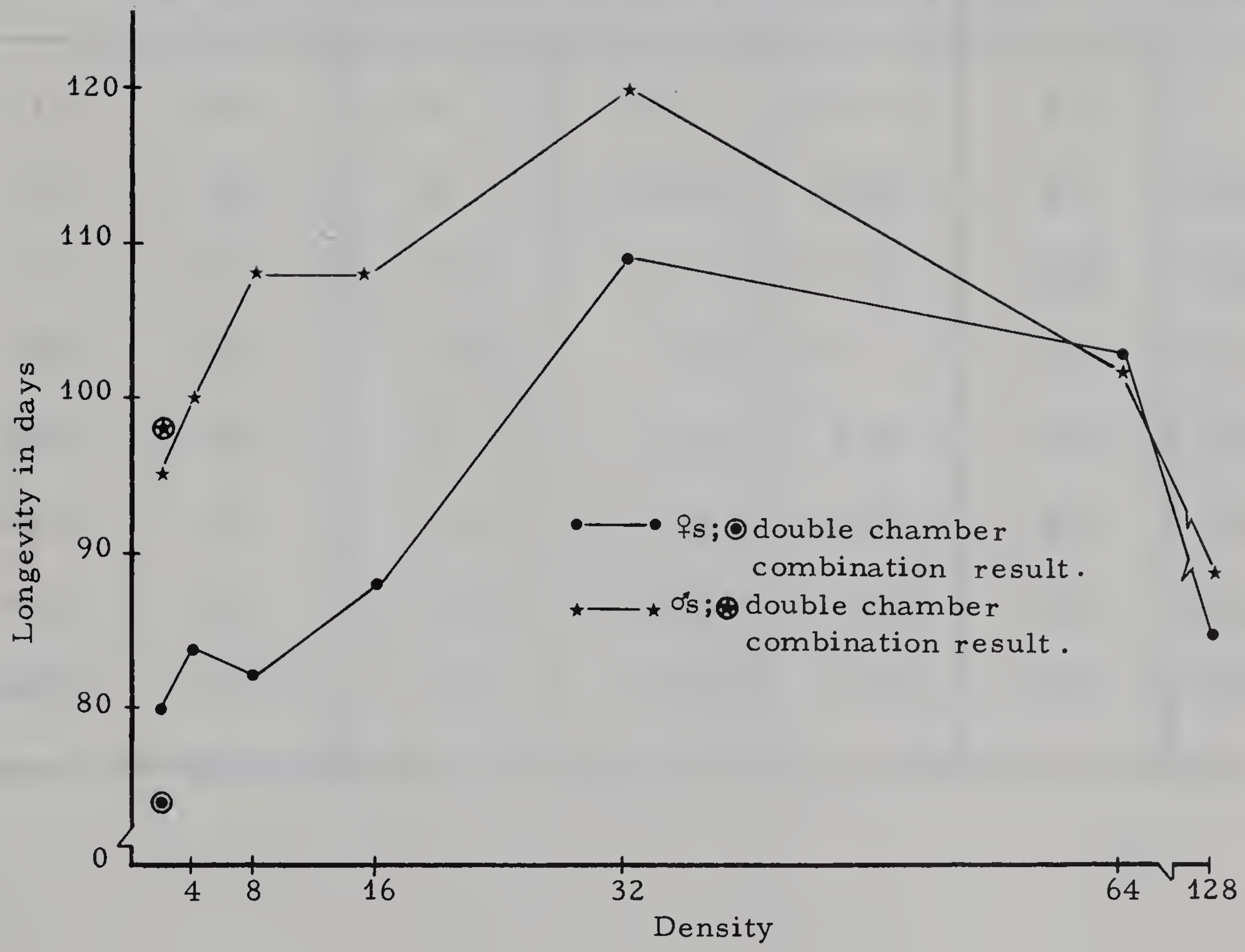


Fig. 26. Effect of the population density of C. lectularius on the longevity of males and females.

[illegible]

Table 11 - Effect of population density on the percentage mortality in the different instars of *C. lectularius*.

Density	Percentage of the total died as					
	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Adult
1	0	0	0	0	0	-
2	0	0	6.6	3.3	0	91.1
4	0	2.5	0	2.5	2.5	92.5
8	0	2.5	2.5	0	0	95.0
16	0	0	6.2	9.4	3.1	81.3
32	0	5.0	6.2	5.0	5.0	78.8
64	0	5.5	11.7	6.3	2.3	74.2
128	1.6	9.4	18.0	9.4	6.2	55.4

and Putnam (1934). However, Wada (1965) claimed that the situation seemed to be more complex and that neurophysiological processes might be involved. Duration of the nymphal stadia and the preoviposition period in the females decreased with the increase of population density. It is difficult from these experiments to specify the manner in which population density produces its effect through increased mutual contact. It seems that increasing the population density may increase the temperature of the microclimate as C. lectularius is always found in aggregates. This increase in the temperature may cause the decreases in the duration of the nymphal stadia and the preoviposition period. Neurophysiological processes may also be involved, but more experiments are required to prove that. Such experiments should relate population density to endocrine activity, moulting, and reproductive functions. Although the number of eggs laid per female increased with the increase in population density to a maximum when the density was 64, it seems that this effect on fecundity resulted from the effect of population density on longevity of the adult stage rather than the effect of population density on fecundity, since there was no significant difference in the number of eggs laid per female per day at the different population densities.

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Appendix

Table 12a - Effect of the size of a single blood meal on the percentage of the first instar nymphs moulting and longevity, at 80 F and 75% R.H. *C. lectularius*

Feeding period in seconds	Average weight of blood meal	% moulted	Longevity in days
Unfed	0	0	11.2±1.1* (20) 4 - 19
15	0.027	0	11.6±0.7 (20) 3 - 15
30	0.054	0	16.6±1.4 (20) 4 - 29
60	0.108	30	19.2±1.4 (20) 8 - 33
120	0.216	100	34.9±1.4 (20) 23 - 39
147(Engorged)	0.260	100	34.9±1.4 (20) 28 - 54
Correlation coefficient r		0.945	0.943
Regression coefficient b			103.87
Chi square x^2			47.90

* Mean ± S.E.
(number of insects)
Range

Table 12b - Effect of the size of a single blood meal on the percentage of second instar nymphs moulting and longevity, at 80 F and 75% R.H. *C. lectularius*

Feeding period in seconds	Average weight of blood meal in mg	% moulted	Longevity in days
Unfed	0	0	35.0±0.56* (20) 32 - 41
15	0.046	0	35.75±1.38 (20) 28 - 49
30	0.092	0	47.55±1.89 (20) 36 - 69
60	0.184	20	57.7±2.42 (20) 40 - 79
120	0.368	35	61.35±1.38 (20) 46 - 79
157(Engorged)	0.481	100	63.65±1.59 (20) 48 - 78
Correlation coefficient r		0.916	0.90
Regression coefficient b			60.63
Chi square x^2			74.37

* Mean ± S.E.
(number of insects)
Range

Table 12c - Effect of the size of a single blood meal on the percentage of third instar nymphs moulting and longevity, at 80 F and 75% R.H. *C. lectularius*

Feeding period in seconds	Average weight of blood meal in mg	% moulted	Longevity in days
Unfed	0	0	37.95±2.36[*] (20) 21 - 57
20	0.124	0	48.6±2.33 (20) 35 - 63
40	0.248	10	52.7±2.02 (20) 40 - 73
80	0.496	40	58.4±1.71 (20) 40 - 68
160	0.992	100	58.8±2.04 (20) 38 - 72
175(Engorged)	1.087	100	63.95±1.71 (20) 48 - 73
Correlation coefficient r		0.99	0.87
Regression coefficient b			17.808
Chi square x^2			24.88

[*] Mean ± S.E.
(number of insects)
Range

Table 12d - Effect of the size of a single blood meal on the percentage of fourth instar nymphs moulting and longevity, at 80 F and 75% R.H. *C. lectularius*

Feeding period in seconds	Average weight of blood meal in mg	% moulted	Longevity in days
Unfed	0	0	52.1±2.16* (20) 39 - 70
30	0.352	0	59.2±1.6 (20) 45 - 69
60	0.704	0	66.2±0.99 (20) 56 - 73
120	1.408	30	68.15±1.56 (20) 43 - 78
243(Engorged)	2.856	100	68.4±1.06 (20) 56 - 73
Correlation coefficient r		0.999	0.774
Regression coefficient b			4.825
Chi square x^2			69.16

* Mean ± S.E.
(number of insects)
Range

Table 12e - Effect of the size of a single blood meal on the percentage of fifth instar nymphs moulting and longevity at 80 F and 75% R.H. *C. lectularius*

Feeding period in seconds	Average weight of blood meal in mg	% moulted	Longevity in days
Unfed	0	0	72.05±1.35* (20) 51 - 80
60	1.086	0	73.60±1.92 (20) 50 - 80
120	2.172	10	79.15±1.77 (20) 55 - 85
240	4.345	70	86.75±2.53 (20) 59 - 97
306(Engorged)	5.533	100	85.55±2.61 (20) 60 - 94
Correlation coefficient r		0.963	0.964
Regression coefficient b			2.867
Chi square x^2			2.18

* Mean ± S.E.
(number of insects)
Range

Table 13 - Effects of the size of blood meals of males and females on the preoviposition period, percentage of females laying eggs, and the number of eggs laid per female. C. lectularius

Experiment number	Female's blood meal		Male's blood meal		Preoviposition period	% females laying eggs	No. of eggs laid per female	% infertile eggs
	period in seconds	wt in mg	period in seconds	wt in mg				
1	Unfed	-	Engorged	6.225	-	0	0	-
2	60	1.446	Engorged	6.225	8.0±0.73 * (6) 6 - 11	30	1.15±0.03 * (6) 1 - 2	4
3	120	2.891	Engorged	6.225	6.2±0.13 (10) 6 - 7	50	2.4±0.48 (10) 1 - 4	1
4	240	5.782	Engorged	6.225	5.33±0.17 (18) 5 - 6	100	5.56±0.47 (20) 3 - 8	4
5	Engorged	6.480	Engorged	6.225	5.5±0.13 (20) 4 - 7	100	11.55±0.62 (20) 5 - 16	4
6	Engorged	6.480	Unfed	-	5.5±0.01 (20) 5 - 6	100	10 (20) 4 - 13	55.5
7	Engorged	6.480	60	1.302	5.55±0.01 (20) 4 - 7	100	10.5 (20) 5 - 14	50
8	Engorged	6.480	120	2.604	5.55±0.02 (20) 4 - 7	100	11 (20) 4 - 16	14
9	Engorged	6.480	240	5.209	5.4±0.02 (20) 4 - 7	100	12.2 (20) 6 - 16	2.5

* Mean ± S.E. (number of insects) range

Table 14 - Summary of the statistical analysis of the data in Table 13.

	Experiments	Correlation coefficient r	Regression coefficient b	Chi square X^2
Female's blood meal and preoviposition period.	2 to 5	-0.908	-0.466	49.80
Female's blood meal and % females laying eggs.	1 to 5	0.994		
Female's blood meal and number of eggs laid per female.	1 to 5	0.929	1.563	49.58
Male's blood meal and % infertile eggs.	5 to 9	-0.923		

Table 15 - Effect of the size of a single blood meal after the final moult on longevity of the adults female and male of C. lectularius.

Feeding period in seconds	Average wt of blood meal in mg	Female Longevity in days			Male	
		Females which did not lay eggs	Females which laid eggs	All females	Average wt of blood meal in mg	Longevity in days
Unfed	-	30.6±2.5* (20) 19 - 64	-	30.6±2.5* (20) 19 - 64	-	31.1±0.9 (20) 27 - 45
60	1.47	33.3±2.0 (12) 27 - 53	26.8±2.3* (6) 20 - 34	31.1±1.7 (18) 20 - 53	1.30	34.1±1.0 (20) 27 - 45
120	2.89	37.1±1.9 (10) 31-50	28.6±1.3 (10) 23 - 36	32.9±1.5 (20) 23 - 50	2.60	37.2±1.2 (20) 28 - 60
240	5.78	-	40.3±1.1 (20) 33 - 52	40.3±1.1 (20) 33 - 52	5.20	42.9±1.8 (20) 29 - 70
Engorged	6.48	-	39.6±4.9 (20) 21 - 67	39.6±4.9 (20) 21 - 67	6.23	43.4±1.0 (20) 30 - 70
Correlation coefficient r		0.99	0.91	0.97		0.99
Regression coefficient b		2.25	2.922	1.707		2.05
Chi square x^2		0.024	3.05	1.77		0.44

* Mean ±S.E.
(number of insects)
range

Table 16a - Effect of the duration and frequency of the blood meal on the first instar of C. lectularius.

Feeding period in seconds	Interval between meals in days	No. of meals between hatching and moult I	No. moulted	Age at moulting in days *	Period in days between last meal and moulting *	Duration of first stadium *	No. died as first instar	Age at death in days *
15	2	3	1	9	4	8	13	27 (8-40)
		4	1	11	4	10		
		5	3	13	4	12		
		6	1	16	5	15		
	4	-	0	-	-	-	20	26 (9-48)
	8	-	0	-	-	-	20	23.4 (9-37)
	16	-	0	-	-	-	20	12.9 (5-18)
60	2	1	6	6.2 (6-7)	5.2 (5-6)	5.2 (5-6)	0	-
		2	8	8.3 (8-9)	5.3 (5-6)	7.3 (7-8)		
		3	6	11	5	10		
	4	1	6	5.2	4.2	4.2	0	-
		2	8	9.4 (9-10)	4.4 (4-5)	8.4 (8-9)		

Cont'd.

Table 16a - Continued

Feeding period in seconds	Interval between meals in days	No. of meals between hatching and moult I	No. moulted	Age at moulting in days*	Period in days between last meal and moulting*	Duration of first stadium*	No. died as first instar	Age at death in days*
60	4	3	5	13.6 (13-14)	4.6 (4-5)	12.6		
		4	1	18	5	17		
	8	1	8	6.3 (6-7)	5.3 (5-6)	5.3 (5-6)	1	96
		2	8	13.6 (13-14)	4.6 (4-5)	12.6 (12-13)		
		3	2	21	4	20		
		6	1	51	10	50		
	16	1	9	6.4 (6-8)	5.4 (4-7)	5.4 (5-7)	9	41.2
		2	2	21	4	20		
120	2	1	20	5.5 (5-6)	4.5 (4-5)	4.5 (4-5)	0	-
	4	1	20	5.5 (5-7)	4.5 (4-6)	4.5 (4-6)	0	-
	8	1	20	5.6 (5-6)	4.6 (4-5)	4.6 (4-5)	0	-
	16	1	20	5.7 (5-7)	4.7 (4.6)	4.7 (4-6)	0	-

Cont'd.

Table 16a - Continued

Feeding period in seconds	Interval between meals in days	No. of meals between hatching and moult I	No. moulted	Age at moulting in days *	Period in days between last meal and moulting *	Duration of first stadium *	No. died as first instar	Age at death in days *
Engorged	2	1	40	4.7 (4-6)	3.7 (3-5)	3.7 (3-5)	0	-
	4	1	40	5.1 (4-6)	4.1 (3-5)	4.1 (3-5)	0	-
	8	1	40	4.6 (4-6)	3.6 (3-5)	3.6 (3-5)	0	-
	16	1	40	4.6 (4-5)	3.6 (3-4)	3.6 (3-4)	0	-

* Average
(Range)

Table 16b - Effect of the duration and frequency of the blood meal on the second instar of C. lectularius.

Feeding period in seconds	Interval between meals in days	No. of meals between moult I and moult II	No. moulted	Age at* moulting in days	Period in days* between last meal and moulting	Duration of* second stadium	No. died as second instar	Age at* death in days
15	2	-	0	-	-	-	7	41.6 (33-47)
60	2	1	3	15.3 (13-17)	7.7 (7-8)	7.3 (7-8)	0	-
		2	11	16.1 (15-19)	6.4 (6-8)	8 (7-9)		
		3	5	18.4 (16-21)	6.2 (6-7)	9.6 (9-10)		
		4	1	22	7	11		
	4	1	2	14 (12-16)	6.5 (6-7)	7	4	47 (42-52)
		2	8	21.25 (15-23)	6.3 (6-7)	9.75 (9-11)		
		3	2	23.5 (20-27)	5.5 (5-6)	16 (15-17)		
		4	4	27 (23-35)	5.75 (5-6)	17.5 (17-18)		
	8	2	2	22.5 (22-23)	5.5 (5-6)	14.5 (14-15)	9	71.3 (36-139)

Cont'd.

Table 16b - Continued

Feeding period in seconds	Interval between meals in days	No. of meals between moult I and moult II	No. moulted	Age at* moulting in days	Period in days* between last meal and moulting	Duration of* second stadium	No. died as second instar	Age at* death in days
60	8	3	1	31	6	24		
		4	6	46 (37-50)	6.1 (4-8)	40.5 (30-63)		
		8	1	89	4	68		
	16	-	0	-	-	-	11	35.8 (14-54)
120	2	1	20	11.8 (11-14)	6.7 (6-7)	7.2 (7-8)	0	-
	4	1	20	12.8 (12-15)	7 (6-8)	7.4 (7-9)	0	-
	8	1	20	12.1 (11-15)	6.3 (6-7)	7.4 (7-9)	0	-
	16	1	20	23	6	17.4 (16 - 18)	0	-
Engorged	2	1	40	11.1 (10-12)	5.1 (4-6)	6.7 (6-7)	0	-
	4	1	40	11.2 (10-14)	5.1 (5-6)	7.1 (6-9)	0	-
	8	1	40	14.3 (14-15)	5.3 (5-6)	9.7 (8-11)	0	-
	16	1	40	23.2 (22-25)	5.6 (5-7)	19.4 (18-22)	0	-

Table 16c - Effect of the duration and frequency of the blood meal on the third instar of *C. lectularius*.

Feeding period in seconds	Interval between meals in days	No. of meals between moult II and III	No. moulted	Age at* moulting in days	Period in days* between last meal and moulting	Duration of* third stadium	No. died as third instar	Age at* death in days
60	2	1	4	22 (20-24)	6.75 (6-7)	6.8 (6-7)	4	95.3 (72-115)
		2	8	24.5 (23-25)	6	8		
		3	3	27.7 (27-29)	6	10		
		4	1	32	5	11		
	4	1	1	19	6	7	7	86.7 (72-110)
		2	9	32.3 (27-35)	6	11.3 (11-12)		
		3	3	41.7 (39-43)	6	16		
	8	2	2	38	5	15.5 (15-16)	5	78.6 (58-120)
		3	2	61.5 (54.69)	5	23.5 (23-24)		
		4	1	69	5	32		
120	2	1	16	17.4 (17-18)	6.4 (6-7)	7	0	-
		2	4	20.5 (19-21)	6	7		

Cont'd.

Table 16c - Continued

Feeding period in seconds	Interval between meals in days	No. of meals between moult II and III	No. moulted	Age at* moulting in days	Period in days* between last meal and moulting	Duration of* third stadium	No. died as third instar	Age at* death in days
120	4	1	14	19.1 (19-20)	6.1 (6-7)	7	0	-
		2	6	25.7 (23-27)	6	11.3 (10-12)		
	8	1	11	23	6	11.5 (11-12)	0	-
		2	9	31	6	18.2 (16-20)		
	16	1	6	39	6	16	2	107 (94-120)
		2	12	54	5	31		
Engorged	2	1	40	16.6 (15-19)	4.4 (3-5)	5.4 (4-6)	0	-
	4	1	40	18.0 (14-22)	5.8 (5-7)	6.5 (6-8)	0	-
	8	1	40	22.7 (22-23)	5.7 (5-6)	8.2 (7-9)	0	-
	16	1	40	38.7 (38-39)	5.7 (5-6)	15.5 (14-17)	0	-

Table 16d - Effect of the duration and frequency of blood meal on the fourth instar of *C. lectularius*.

Feeding period in seconds	Interval between meals in days	No. of meals between moult III and IV	No. moulted	Age at* moulting in days	Period in days* between last meal and moulting	Duration of* fourth stadium	No. died as fourth instar	Age at* death in days
60	2	2	1	29	7	9	7	85.1
		3	4	33 (32-34)	7	10.2 (10-11)		
		4	4	35.5 (35-36)	7.5 (7-8)	12.5 (12-13)		
	4	2	1	32	7	13	8	86.8 (45-110)
		3	4	48 (44-52)	7	17		
	8	-	0	-	-	-	5	148.8 (140-156)
120	2	1	7	23	6	7	0	-
		2	11	25.5 (25-29)	6	7.5 (7-8)		
		3	2	31	6	10		
	4	1	6	28	7	9	4	111.5 (95-122)
		2	6	32.3 (31-35)	6	11.7 (11-12)		
		3	4	36	7	17		

Cont'd.

Table 16d - Continued

Feeding period in seconds	Interval between meals in days	No. of meals between moult III and IV	No. moulted	Age at moulting in days*	Period in days between last meal and moulting*	Duration of fourth stadium*	No. died as fourth instar	Age at death in days*
120	8	1	3	34.7 (32-40)	7	9	12	105.4 (84-120)
		2	3	41.7 (39-47)	6	16		
		3	2	48	7	25		
	16	1	2	56	7	17	14	93.6 (84-103)
		2	2	79 (71-87)	7.5 (7-8)	32.5 (32-33)		
Engorged	2	1	40	22.8 (21-24)	5.4 (4-6)	6.4 (5-7)	0	-
	4	1	40	25 (20-27)	5.8 (4-7)	8.1 (4-10)	0	-
	8	1	40	30.7 (30-31)	5.7 (5-6)	8.1 (7-9)	0	-
	16	1	40	54.7 (54-55)	6.4 (5-7)	16.5 (15-17)	0	-

Table 16e - Effect of the duration and frequency of the blood meal on the fifth instar of _C_. _lectularius_.

Feeding period in seconds	Interval between meals in days	No. of meals between moult IV and V	No. moulted	Age at moulting in days*	Period in days between last meal and moulting*	Duration of fifth stadium*	No. died as fifth instar	Age at death in days*
60	2	3	3	43.7 (41-46)	8.7 (8-9)	12.3 (12-13)	6	116.7 (99-144)
	4	-	0	-	-	-	5	93.4 (85-107)
120	2	1	3	32.7 (32-34)	9	9	6	128.8 (115-140)
		2	6	33.3 (33-35)	8	10		
		3	4	37	8	12		
		4	1	40	7	13		
	4	1	2	37	8	9	8	112.3 (98-125)
		2	4	42.5 (40-45)	7.5 (7-8)	13 (12-14)		
		3	2	44	7	16		
	8	1	1	42	9	10	5	96.4 (84-111)
		2	2	55	6	16		
	16	-	0	-	-	-	5	90.6 (80-98)

Table 16e - Continued

Feeding periods in seconds	Interval between meals in days	No. of meals between moult IV and V	No. moulted	Age at moulting in days*	Period in days between last meal and moulting*	Duration of fifth stadium*	No. died as fifth instar	Age at death in days*
240	2	1	15	28	7 (6-8)	6.7 (6-7)	0	-
		2	5	31.2 (30-33)	5.4 (5-6)	6.4 (6-7)		
	4	1	14	31.3 (27-34)	6.5 (6-8)	7 (6-9)	0	-
		2	6	37 (34-40)	5.3 (5-7)	10.5 (9-12)		
	8	1	11	38.5 (38-39)	5.5 (5-6)	9	0	-
		2	9	46.6 (46-47)	5.6 (5-6)	15.6 (15-16)		
	16	1	9	70.6 (70-71)	6.6 (6-7)	16	1	120
		2	10	85.9 (85-86)	5.9 (5-6)	31		
Engorged	2	1	20	28 (26-31)	4.2 (4-5)	6.2 (6-7)	0	-
	4	1	20	31.4 (27-34)	5.4 (5-7)	6.5 (6-8)	0	-
	8	1	20	38.1 (37-39)	5.1 (4-6)	7.4 (7-8)	0	-
	16	1	20	69.4 (69-71)	4.6 (4-5)	14.7 (14-17)	0	-

Table 17 - Effect of the size and frequency of blood meals on weight changes during development in C. lectularius.

Feeding period in seconds	Interval between meals in days	Instar I to II			Instar II to III			Instar III to IV			Instar IV to V			Instar V to ♀			Instar V to ♂		
		(i)*	(ii)**	(iii)***	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
15	2	0.13	# 0.03 (6)	0.24	-	# -	-	-	# -	-	-	# -	-	-	# -	-	-	# -	-
60	2	0.23	0.05 (20)	0.23	0.52	0.12 (20)	0.23	0.78	0.17 (16)	0.22	2.39	0.56 (9)	0.23	3.33	1.00 (2)	0.30	3.36	0.81 (1)	0.24
	4	0.22	0.05 (20)	0.23	0.59	0.12 (16)	0.20	0.82	0.16 (13)	0.20	1.97	0.49 (5)	0.25	-	-	-	-	-	-
	8	0.20	0.04 (19)	0.20	0.92	0.10 (10)	0.11	1.04	0.15 (5)	0.14	-	-	-	-	-	-	-	-	-
	16	0.13	0.03 (11)	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
120	2	0.22	0.08 (20)	0.37	0.47	0.18 (20)	0.38	0.89	0.23 (20)	0.26	2.53	0.80 (20)	0.32	4.66	1.31 (8)	0.36	5.15	1.15 (6)	0.22
	4	0.22	0.07 (20)	0.32	0.47	0.16 (20)	0.34	0.97	0.23 (20)	0.24	2.68	0.80 (16)	0.30	4.44	1.17 (5)	0.38	4.48	1.05 (3)	0.23
	8	0.22	0.06 (20)	0.28	0.47	0.15 (20)	0.32	1.12	0.22 (20)	0.20	2.70	0.70 (8)	0.26	3.33	0.94 (2)	0.35	4.50	1.04 (1)	0.23
	16	0.22	0.06 (20)	0.28	0.47	0.14 (20)	0.30	1.26	0.20 (18)	0.16	1.69	0.63 (4)	0.37	-	-	-	-	-	-
240	2	-	-	-	-	-	-	-	-	-	-	-	-	6.66	1.64 (11)	0.25	4.93	1.21 (9)	0.25

Cont'd.

Table 17 - Continued

Feeding period in seconds	Interval between meals in days	Instar I to II			Instar II to III			Instar III to IV			Instar IV to V			Instar V to ♀			Instar V to ♂		
		(i)*	(ii)**	(iii)***	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
240	4	-	-	-	-	-	-	-	-	-	-	-	-	6.22	1.51 (10)	0.24	5.38	1.08 (10)	0.20
	8	-	-	-	-	-	-	-	-	-	-	-	-	5.33	1.43 (12)	0.27	5.82	1.03 (8)	0.18
	16	-	-	-	-	-	-	-	-	-	-	-	-	7.10	1.30 (9)	0.18	6.72	0.86 (10)	0.13
Engorgement	2	0.26	0.11 (40)	0.42	0.62	0.25 (40)	0.40	1.08	0.44 (40)	0.41	2.86	1.37 (40)	0.48	5.53	2.26 (13)	0.41	5.42	1.90 (7)	0.35
	4	0.26	0.10 (40)	0.38	0.62	0.24 (40)	0.39	1.09	0.34 (40)	0.31	2.81	1.29 (40)	0.46	5.64	2.03 (11)	0.36	5.38	1.73 (9)	0.32
	8	0.26	0.10 (40)	0.38	0.62	0.20 (40)	0.32	1.09	0.32 (40)	0.29	2.88	1.12 (40)	0.39	5.49	1.92 (10)	0.35	5.53	1.52 (10)	0.27
	16	0.26	0.10 (40)	0.38	0.62	0.18 (40)	0.29	1.09	0.26 (40)	0.24	2.83	1.00 (40)	0.35	5.86	1.80 (9)	0.32	5.48	1.25 (11)	0.23

* (i)	Average weight of blood meals in mg.
**(ii)	Average difference in body weight in mg.
***(iii)	Ratio of difference in body weight to weight of blood meals.
#	Average (no. of insects)

Table 18 - Effects of the size and frequency of blood meals on preoviposition period, fecundity and longevity of the female C. lectularius.

Feeding period in seconds	Interval in days	No. of females	Aver. body wt in mg.	No. of blood meals during preoviposition period	Preovi-position period in days	Eggs per female	No. of meals during adult life	Eggs/mg of blood	Average longevity of female in days	Eggs per day
60	2	2	1.97	4	11	38	20	1.3	50	0.76
120	2	8	2.67	2	9	85	19	1.5	67	1.27
	4	5	2.50	2 3	16 18	40	12	1.2	54	0.74
	8	2	2.14	2	19	14	6	0.81	42	0.33
240	2	11	3.88	1	5.5	160	19	1.5	84	1.90
	4	10	3.55	1	5.3	138	18	1.3	80	1.73
	8	12	3.24	1	6.9	62	8	1.3	61	1.02
	16	9	2.91	1	8	24	3	1.38	49	0.49
Engorged (290)	2	13	4.50	1	5.5	185	20	1.4	83	2.22
	4	11	4.07	1	5.5	171	17	1.5	80	2.14
	8	10	3.73	1	7	80	9	1.37	67	1.19
	16	9	3.41	1	8	37	4	1.4	52	0.71

Table 19 - Effects of the size and frequency of blood meals on preoviposition period, fecundity, and longevity of females C. lectularius taken from the standard culture.

Feeding period in seconds	Interval in days	Aver. no. of blood meals during preoviposition period	Aver. preoviposition period in days	Average eggs/ female	No. of blood meals during adult life	Eggs/mg of blood	Average longevity in days	Eggs/day
15	2	-	-	-	16	-	34	-
	4	-	-	-	7	-	27	-
	8	-	-	-	3	-	20	-
	16	-	-	-	1	-	18	-
30	2	6	14	9.5	21	0.8	44	0.22
	4	4	16	3	9	0.6	38	0.08
	8	-	-	-	5	-	40	-
	16	-	-	-		-	27	-
60	2	4	10	46	29	1.4	60	0.77
	4	4	15	14	12	1.0	49	0.29
	8	2	18	4.5	5	0.8	35	0.13
	16	2	21	1	2	0.3	24	0.04
120	2	3	8	113	33	1.5	72	1.57
	4	3	10	44	15	1.3	61	0.72
	8	2	14	8	4	0.9	48	0.16
	16	2	20	2	2	0.4	32	0.06

Table 20 - Statistical analysis of some of the data in Tables 18 and 19.

	Correlation coefficient r	
	Data in Table 18	Data in Tables 18 and 19
Weight of blood meals and eggs per female	0.99	0.99
Weight of blood meals and eggs per mg of blood	0.45	0.70
Body weight and eggs per female	0.32	-
Longevity and eggs per female	0.97	0.94
Weight of blood meals and longevity of females	0.97	-

Table 21 - Effects of the size and frequency of blood meals on the longevity of males and the percentage of infertile eggs laid by the mated females.

Feeding period in seconds	Interval between blood meals in day	Number of males	Average no. of blood meals	Average total wt of blood meals in mg	Average longevity in days	% infertile eggs laid by females
60	2	1	26	33.85	55	35
120	2	6	35	91.14	72	8
	4	3	15	39.06	60	7
	8	1	6	15.62	48	20
240	2	9	45	234.36	91	2
	4	10	20	104.16	82	3
	8	8	8	41.66	66	3
	16	11	4	20.83	53	5
Engorged (287)	2	7	46	286.58	98	1
	4	9	22	137.06	88	1
	8	10	9	56.07	71	2
	16	11	4	24.92	51	4

Table 22 - Effects of the size and frequency of blood meals on the longevity of males and the percentage of infertile eggs laid by the mated females. (Insects taken from the standard culture)

Feeding period in seconds	Interval between blood meals in day	Average number of blood meals	Average total weight of blood meals in mg	Average longevity in days	% infertile eggs laid by females
15	2	15	4.88	33	-
	4	7	2.28	30	-
	8	3	0.98	21	-
	16	1	0.33	17	-
30	2	21	13.67	44	40
	4	10	6.51	40	45
	8	5	3.26	38	-
	16	2	1.30	27	-
60	2	33	42.97	68	30
	4	13	16.93	54	32
	8	5	6.51	42	50
	16	2	2.60	30	60
120	2	38	98.95	81	7
	4	17	44.68	69	9
	8	7	18.23	54	16
	16	3	7.81	42	28

Table 23 - Protein content in the different instars of C. *lectularius*.

Instar	Number of insects used	Average wt in mg	Protein content/ insect in mg*	% Protein
Egg	30	0.15	0.034 (3) 0.031-0.037	22.4 (3) 20.6-24.6
First	45	0.10	0.022 (3) 0.020-0.023	21.8 (3) 20.0-23.0
Second	30	0.25	0.057 (3) 0.052-0.060	22.8 (3) 20.9-24.2
Third	30	0.50	0.127 (3) 0.119-0.134	25.4 (3) 22.3-28.1
Fourth	12	0.77	0.195 (3) 0.173-0.209	25.3 (3) 23.3-26.8
Fifth	10	1.63	0.451 (5) 0.442-0.502	27.7 (5) 25.4-28.7
Female	10	3.13	0.569 (10) 0.558-0.579	18.2 (10) 17.4-19.3
Female**	10	4.04	1.054 (10) 1.039-1.068	26.1 (10) 23.3-28.4
Male	10	2.90	0.736 (10) 0.718-0.754	25.4 (10) 22.3-27.6
Male**	10	3.50	0.882 (10) 0.859-0.895	27.2 (10) 26.3-28.8

* Average
(number of estimations)
range

** Insects kept separated from the opposite sex.

Table 24 - Oxygen consumption, carbon dioxide output, and respiratory quotient in the different instars of C. lectularius.

Instar	Average body wt in mg	ul O_2/ insect/ hour	ul O_2/ mg/hour	ul CO_2 insect/ hour	R.Q.
First	0.40	0.02	0.05	0.019	0.95
Second	0.74	0.04	0.05	0.036	0.90
Third	1.27	0.07	0.06	0.063	0.90
Fourth	2.00	0.20	0.10	0.182	0.91
Fifth	5.15	0.57	0.11	0.502	0.88
Female	6.04	0.88	0.15	0.792	0.90
Male	5.20	0.60	0.12	0.528	0.88

Table 25 - Effect of population density of *C. lectularius* on the duration of the nymphal stadia.

Density	Replicates	Duration of				
		First stadium	Second stadium	Third stadium	Fourth stadium	Fifth stadium
1	20	3.7 (3-4)	6.7 (5-7)	5.4 (5-6)	6.4 (6-7)	6.2 (6-7)
2	30	3.8 (3-5)	7.0 (5-8)	5.5 (5-6)	6.5 (6-7)	6.5 (6-7)
4	20	3.7 (3-4)	6.2 (5-7)	5.4 (5-6)	6.1 (5-7)	6.2 (5-7)
8	15	4.0 (3-5)	6.4 (5-7)	5.0 (4-6)	6.2 (5-7)	6.0 (5-7)
16	10	3.6 (3-4)	5.3 (4-6)	5.1 (4-6)	6.0 (5-7)	5.0 (4-6)
32	5	3.6 (3-4)	4.0 (3-5)	4.0 (3-5)	5.1 (4-6)	4.7 (4-5)
64	2	3.7 (3-4)	4.1 (3-5)	4.1 (3-5)	5.1 (4-6)	4.0 (3-5)
128	2	4.0 (3-5)	4.0 (3-5)	3.7 (3-4)	5.2 (4-6)	4.3 (3-5)
1**	20	3.9 (3-5)	6.8 (6-8)	5.3 (5-6)	6.2 (6-7)	6.4 (6-7)

* Average in days
(Range)

** Experiment using the double chamber combination.

Table 26 - Effects of the population density of *C. lectularius* on the preoviposition period, number of eggs laid per female, and the longevity of males and females.

Density	Minimum preoviposition period in days	Number of eggs/ female	Longevity in days		Eggs/day
			Females	Males	
2	6* (6)	179* (159 - 184)	80* (75 - 86)	95* (88 - 105)	2.2
4	6 (5 - 7)	174 (149 - 188)	84 (77 - 88)	100 (91 - 112)	2.1
8	5 (4 - 6)	182 (164 - 192)	82 (78 - 87)	108 (101- 114)	2.2
16	5 (4 - 6)	191 (174 - 212)	88 (81 - 96)	108 (99 - 116)	2.2
32	4 (4)	224 (208 - 232)	109 (91 - 116)	120 (111- 129)	2.1
64	4 (4)	229 (209 - 249)	103 (90 - 109)	102 (91 - 113)	2.2
128	4 (4)	198 (184 - 212)	85 (76 - 94)	89 (79 - 99)	2.4
2**	6 (5 - 7)	170 (152 - 178)	74 (61 - 79)	98 (82 - 108)	2.3

* Average
(Range)

** Experiment using the double chamber combination

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